Aus der Klinik für Klinik für Dermatologie, Venerologie und Allergologie
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DISSERTATION

Study of the efficacy of a new topical skin formulation rich in antioxidants in patients with mild to moderate atopic dermatitis

Studie über die Wirksamkeit einer neuen, antioxidantienreichen topischen Hautrezeptur bei Patienten mit leichter bis mittelschwerer Atopic Dermatitis

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List of abbreviations

AD·····atopic dermatitis

EPR·····electron paramagnetic resonance

LOR·····loricrin

FLG·····filaggrin

IVL·····involucrin

IL·····interleukin

NMF·····natural moisturizing factor

Th2·····T-helper 2

TCS·····topical corticosteroids

TCI·····topical calcineurin inhibitors

ROS·····reactive oxygen species

AOS·····antioxidant system

STAT6·····signal transducer and activator of transcription 6

CCL26·····CC-chemokine ligand 26

NHEKs·····pooled primary normal human epidermal keratinocytes

CA2·····carbonic anhydrase II

COL1A1·····collagen type I alpha 1 chain

TNF-α·····tumor necrosis factor alpha

RPF·····Radical Protection Factor

DPPH·····2,2-Diphenyl-1-picrylhydrazyl

CRM·····confocal Raman micro spectroscopy

VAS·····visual analogue scale

TEWL·····transepidermal water loss

Abstract

Atopic dermatitis (AD) is a common chronic inflammatory skin disease. Its pathogenic mechanism is complex, in which oxidative stress plays an important pathogenic role. In the treatment strategy of atopic dermatitis, the role of topical preparations cannot be ignored. As a chronic relapsing inflammatory disease, although topical steroids and immunomodulators can relieve symptoms relatively quickly during the acute period, they cannot be used safely for a long time due to their side effects. Although emollients can be safely used for a long time, they are usually used as an adjuvant therapy, and their independent efficacy studies in vivo are very limited. Given the above information, a topical formulation that is effective in the acute phase and can act as an emollient for long-term use would greatly improve the quality of life of AD patients. Nevertheless, the skin is an organ with barrier properties, and the bio permeable activity of topically applied substances often affects the efficacy of topical formulations. Therefore, to develop a new emollient for long-term use, the work was carried out in three steps: 1. In view of the anti-inflammatory and antioxidant effects of green tea extract, apple extract and curly kale extract reported in related publications, the above extracts were mixed and the anti-inflammatory and skin barrier repairing effects were investigated in vitro, 2. the penetration depth of a formulation containing the extract mixture was investigated on porcine skin ex vivo using Raman microscopy. 3. the effect of the antioxidant-rich formulation was investigated in a clinical trial on skin lesions in patients with mild to moderate AD. An in vitro investigation on cells clearly showed the increase in barrier related and anti-inflammatory properties. The antioxidant capacity of the extracts quantified by electron paramagnetic resonance (EPR) spectroscopy, confirmed the strong antioxidant effects of the extracts. The penetration results showed that cream containing the extract could penetrate through the skin’s stratum corneum and reached the active layer of the skin. Subsequently, in a 4-week randomized controlled clinical trial, the excellent therapeutic effects of the cream containing the extract were observed on lesions of patients with mild to moderate AD. This included an itching and TEWL reduction and an increased skin moisture.

Zusammenfassung

Atopische Dermatitis (AD) ist eine häufige chronisch-entzündliche Hauterkrankung. Ihr Pathogenitätsmechanismus ist komplex, wobei oxidativer Stress eine wichtige pathogene Rolle spielt. Bei der Behandlungsstrategie der atopischen Dermatitis darf die Rolle der topischen Präparate nicht außer Acht gelassen werden. Da es sich um eine chronisch-rezidivierende Entzündungskrankheit handelt, können topische Steroide und Immunmodulatoren die Symptome in der akuten Phase zwar relativ schnell lindern, aber aufgrund ihrer Nebenwirkungen nicht über einen langen Zeitraum hinweg sicher angewendet werden. Obwohl Emollientien über einen langen Zeitraum sicher eingesetzt werden können, werden sie in der Regel als unterstützende Therapie verwendet, und ihre unabhängigen Wirksamkeitsstudien in vivo sind sehr begrenzt. In Anbetracht der oben genannten Informationen würde eine topische Formulierung, die in der akuten Phase wirksam ist und bei langfristiger Anwendung als Emolliens fungieren kann, die Lebensqualität von AD-Patienten erheblich verbessern. Allerdings ist die Haut ein Organ mit Barriereeigenschaften, und die biopermeable Aktivität topisch applizierter Substanzen beeinträchtigt häufig die Wirksamkeit topischer Formulierungen. Um ein neues Emolliens für die Langzeitanwendung zu entwickeln, wurde die Arbeit daher in drei Schritten durchgeführt: 1. In Anbetracht der entzündungshemmenden und antioxidativen Wirkungen von Grüntee-, Apfel- und Grünkohl-Extrakt, über die in einschlägigen Veröffentlichungen berichtet wurde, wurden die genannten Extrakte gemischt und die entzündungshemmenden und die Hautbarriere reparierenden Wirkungen in vitro untersucht. 2. Die Eindringtiefe einer Formulierung, die die Extraktmischung enthielt, wurde ex vivo auf Schweinehaut mittels Raman-Mikroskopie untersucht. 3. Die Wirkung der an Antioxidantien reichen Formulierung wurde in einer klinischen Studie an Hautläsionen bei Patienten mit leichter bis mittelschwerer AD untersucht. In vitro-Untersuchungen an Zellen zeigen deutlich die Erhöhung der barrierebezogenen und entzündungshemmenden Eigenschaften. Die antioxidative Kapazität der Extrakte, die mit Hilfe der EPR Spektroskopie quantifiziert wurde, bestätigte die starke antioxidative Wirkung der Extrakte. Die Penetrationsergebnisse zeigen, dass eine Creme, die den Extrakt enthält, durch das Stratum corneum dringen und die aktive Schicht der Haut erreichen kann. Anschließend wurden in einer 4-wöchigen randomisierten, kontrollierten klinischen Studie die ausgezeichneten therapeutischen Wirkungen der den Extrakt

enthaltenden Creme auf Läsionen von Patienten mit leichter bis mittelschwerer AD beobachtet. Dazu gehören die Verringerung von Juckreiz und TEWL sowie die Erhöhung der Hautfeuchtigkeit.

1 Introduction

As a common non-specific inflammatory disease of the skin, the complex pathogenesis and diverse clinical manifestations of AD pose a challenge for the diagnosis and treatment of the disease. Its long-term chronic and recurrent clinical features have even further negative impacts on AD patients. For patients with AD of varying severity, the use of moisturizers is an essential and important part in the treatment and the prevention of AD eruptions [2]. At the same time, the use of emollients can reduce the application of prescription agents, including topical corticosteroids, and thus avoid side effects and tachyphylaxis caused by the long-term repeated use of prescription medications [3,4].
Nevertheless, few prescription and non-prescription emollients have been reported to have superior therapeutic effects on AD patients [3].
With the recent research on the pathogenic mechanisms associated with AD, more and more novel medicaments are being used in the therapeutic management of AD. Among these, the efficacy of using vitamin E as an adjunctive treatment for AD based on its antioxidative effects has been demonstrated [5]. Many plants and foods are rich in antioxidants and given the absence of side effects and the abundance of such natural antioxidants, they may serve as a therapeutic option for diseases involving oxidative stress [6]. However, there are few studies related to the therapeutic benefits of topical antioxidant agents for AD patients based on antioxidant stress mechanisms. Given the important role of topical therapy in the treatment of patients with mild to moderate AD, the involvement of oxidative stress in AD progression and the potential risks associated with the long-term use of topical steroids and immunomodulators, a safe and effective topical emollient with antioxidant effect might be essential in an AD treatment strategy.

1.1 Atopic dermatitis

In patients with AD, pruritus is the predominant clinical manifestation and is present in almost all patients [7]. Other clinical manifestations include inflammation, sleep disturbance and dry skin.
The increasing incidence of AD and the negative impact of its clinical manifestations on patients' quality of life have made it the most burdensome disease over acne and psoriasis [8]. AD usually develops in childhood and is more common in women than in men. In

recent years, the incidence of AD in adults is high and presents clinical signs different from those of childhood AD [9].

1.2 Pathogenesis of atopic dermatitis

The pathogenesis of AD is complex and a combination of genetic, immunlogic and environmental factors. Skin barrier dysfunction is the most significant pathological change in AD. The keratinocytes, which start from the basal layer of the skin and continue to differentiate upwards, form a multi-layered barrier of the skin that resists the penetration of environmental allergens and prevents moisture loss. In AD patients, due to impaired terminal differentiation, keratinocytes have reduced synthesis of some molecules that maintain the skin barrier function, such as loricrin (LOR), filaggrin (FLG), and involucrin (IVL)[10].

Mutations in the FLG gene, polymorphisms in the IL(interleukin)-4 receptor and vitamin D receptor are important genetic factors associated with AD [11-13]. Among them, FLG loss-of-function mutations have been studied most frequently.

On the one hand, the loss of FLG function mutation reduces the production of the natural moisturizing factor (NMF) resulting in dryness [14]; on the other hand, the lack of NMF impairs the skin barrier function and more allergens penetrate the skin barrier, causing immune dysregulation [15], which further disrupts the skin barrier function [16].

The involvement of individual immune responses varies among the different subtypes of AD, with type 2 response playing a major role in all clinical phenotypes [17]. IL-31-induced pruritus , the downregulation of FLG and the decreased expression of antibacterial peptides is associated with T-helper 2 (Th2) lymphocytes-mediated overexpression of multiple interleukins and chemokines [16,18].The long-standing type 2 immune response, which means the existing overexpression of IL-13, IL-4 and chemokines such as CCL26, CCL22, leads to impaired skin barrier function and bacterial colonization.

1.3 Treatment strategies for atopic dermatitis

The basic management principle of AD is to improve clinical symptoms in the acute phase and minimize the relapse cycle to achieve long-term disease control [19]. The treatment of AD patients is based on the severity of the disease and the patient's own condition. For patients with mild to moderate AD, topical treatment is effective in relieving skin inflammation, reducing clinical symptoms and controlling the formation of new lesions [20].

At the same time, risk factors such as allergen exposure, alcohol consumption, and stress should be avoided [8]. Topical corticosteroids (TCS) and topical calcineurin inhibitors (TCI) are the most commonly used topical treatment in AD patients, especially in terms of their anti-inflammatory efficacy [21]. Although the effectiveness of TCS in the treatment of AD has been confirmed, long-term use can lead to skin atrophy and disruption of the skin barrier leading to relapse from discontinuation [22,23]. Similarly, based on the potential theoretical risk of malignancy with TCI, they should only be used for short curves of discontinuous treatment [24]. In summary, a new formulation that strikes a clinical equipoise between efficacy and safety is required for long-term management of AD patients.

1.4 Oxidative stress and Atopic Dermatitis

As mentioned before, the negative interaction between persistent Th2 polarization and skin barrier dysfunction leads to the chronicity of AD inflammation. Overproduction of reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), is highly correlated with chronic skin inflammation [25]. When the scavenging capacity of the antioxidant system (AOS) is not sufficient to remove the generated ROS, oxidative stress occurs. Oxidative stress perpetuates the signal transducer and activator of transcription 6 (STAT6) signaling cascade by oxidative inactivation of protein tyrosine phosphatase 1B (PTPN1) [26]. STAT6 signaling downregulates the expression of involucrin and loricrin, which affect the epidermal differentiation, and upregulates the expression of eosinophil chemokines such as CC-chemokine ligand 26 (CCL26) [27,28]. Related studies have demonstrated a positive correlation between eosinophil numbers and AD severity [29]. The role of oxidative stress in the molecular mechanisms involved in the development and maintenance of AD makes it a potential approach for AD management.

1.5 Aims of the study

The aim of this study was to prepare a new skin care product that should be safe and could improve clinical symptoms in AD patients. To achieve this aim, the following hypotheses and measurements were made.

1. Selection of extracts with anti-inflammatory, antioxidant properties

Based on the available literature data, several extracts with anti-inflammatory and antioxidant properties were selected and mixed, assuming that mixing improves anti-inflammatory and antioxidant abilities (joint contribution).

2. The in vitro study of the extracts' anti-inflammatory and antioxidant ability and the skin barrier repair ability

a. In cellular experiments, it was hypothesized that the expression levels of the relevant inflammatory factors in the AD-like model could represent the degree of inflammatory response, and the application of the extract could reduce the expression of AD-related inflammatory factors (external investigation).

b. In cellular experiments, assuming that the expression levels of barrier protein molecules in the in vitro model of AD can reflect the barrier function level, the application of the extract can significantly enhance the expression of barrier proteins in this model (external investigation).

c. EPR is an effective way to quantify the antioxidant capacity of different formulations, and the formulation containing extracts shows a higher antioxidant capacity (own contribution).

3. The penetration efficiency of the extract-enriched formulation was determined by ex vivo experiments (own contribution).

Given the oxidation properties of antioxidants, antioxidant-rich creams should have sufficient penetration efficiency to ensure that the antioxidant components can reach the active layer of the epidermis and remove excess ROS. The penetration concentration curve of a specific substance can be measured by confocal Raman micro spectroscopy. Since the structure of porcine skin is similar to that of human skin, it is assumed that the penetration efficiency of verum cream on porcine skin can reflect its penetration efficiency on normal human skin (own contribution).

4. A clinical study was conducted to verify the effect of the extract-rich formulation on the improvement of symptoms in AD patients (own contribution).

2 Methods

2.1 Materials

2.1.1. Effectiveness of extracts in AD-like in vitro model

The extracts added to the verum cream in this study consisted of the following food-grade commercially available plant extracts: curly kale extract (Anklam Extract GmbH, Anklam, Germany), green tea extract (Eurochem Feinchemie GmbH, Gröbenzell, Germany), apple extract (Herbstreith & Fox KG, Neuenbürg/Württ, Germany) and L-arginin (Roth, Karlsruhe, Germany). Among them, kaempferol in kale extract, catechins in green tea extract, and apple flavonoids in apple extract have been proved to have anti-inflammatory and/or antioxidant effects in previously published data [30-34]. L-arginine was reported to be highly effective in repairing defective skin barrier [35].

2.1.2. Preparations of the two formulations used in this study

The specific ingredients in the placebo cream and the verum cream used in the study are shown in the table 1 and table 2.

Table 1. Components of placebo cream (adapted from Yu Zhang et al., 2022[1]).

100g Placebo cream (DAC Basis Cream)
• 40.0 g puriefied water
• 25.5 g white vaseline (petroleum jelly)
• 10.0 g propylene glycol
• 7.5 g medium-chain trigylcerides (mygliol®812, neutral oil)
• 7.0 g macrogol-20-glycerol monostearate
• 6.0 g cetylalcohol
• 4.0 g glycerol monostearate 60

Table 2. Components of verum cream (adapted from Yu Zhang et al., 2022[1]).

100g Verum cream
• 98.0 g placebo cream
• 0.5 g L-arginine
• 0.5 g curly kale extract (30% kaempferol flavonoids)
• 0.5 g apple extract (15% phlorizin ,5% quercetin flavonoids)
• 0.5 g green tea extract (60% epigallocatechin gallate)

2.2 In vitro research on cell culture

To investigate the potential therapeutic effects of this extract on AD patients, an in vitro AD-model was established using pooled primary normal human epidermal keratinocytes (NHEKs) differentiated by mixed cytokine stimulation. The NHEKs used in this study were isolated from the juvenile foreskin epidermis of three male donors. (Lot Number: 456Z001.1, PromoCell, Heidelberg). Passage-4 NHEKs with Keratinocyte Growth Medium 2 (KGM2) Supplement Mix including $CaCl_2$ (PromoCell) were seeded in a 24-well plate in KGM2 (PromoCell, Heidelberg, Germany). After confluency (90%-100%), cells were cultured for 48h in a medium containing 1.3 mM $CaCl_2$ to induce differentiation. Differentiated NHEKs were mixed with cytokines (IL-22 ,IL-13,IL-4 and tumor necrosis factor alpha (TNF-α) with the same concentration:10 ng/mL) to establish an AD-like inflammation model as described previously [36].

Subsequently, these cells were cultured with a 1:800 dilution of mixed extracts (kale extract, green tea extract, apple extract, L-arginine in 50% ethanol at a concentration of 10 ng/mL) or a carrier control (50% ethanol) at 37°C for 20 hours in a 5% CO_2 environment. After adding the extract or carrier control in the AD-like model, the expression level of AD-related genes were measured by real-time PCR. We used PrimeScript reverse transcriptase kit (Takara Bio, Saint-Germain-en-Laye, France) to transcribe RNA isolated by Crystal RNAmagic kit (Biolabproducts, Bebensee, Germany) to cDNA. Real-time PCR analysis was done using the QuantStudio3 system (Thermo Fisher Scientific, Schwerte, Germany) using the temperature profile described by Roth, S.A. et al.[37]. Gene expression levels of carbonic anhydrase II (CA2), collagen type I alpha 1 chain (COL1A1), Interleukin-24 (IL-24), CC-chemokine ligand 26 (CCL26), filaggrin (FLG), loricrin (LOR), involucrin (IVL), and homo sapiens ribosomal protein L38 (RPL38) were measured. The primers sequences used are shown in Table 3.

Table 3. Primer sequences for real-time PCR to establish AD-related gene expression levels(adapted from Yu Zhang et al., 2022[1]).

Gene	gene acc.no.	Forward Primer	Reverse Primer
IVL	NM_005547.2	5′-GGAGGAGGAACAGTCTTGAGG-3′	5′-CTGCCTCAGCCTTACTGTGA-3′
FLG	NM_002016.1	5′-GGCAAATCCTGAAGAATCCAGATG-3′	5′-GGTAAATTCTCTTTTCTGGTAGACTC-3′
LOR	NM_000427.2	5′-CTCTCCTCACTCACCCTTCCT-3′	5′-AGGTCTTCACGCAGTCCAC-3‘
IL-24	NM_006850.3	5′-GTTCCCCAGAAACTGTGGGA-3′	5′-CGAGACGTTCTGCAGAACC-3′
CCL26	NM_006072.4	5′-AATTGAGGCTGAGCCAAAGA-3′	5′-ATCAGGCCCTTCTCAGGTTT-3′
COL1A1	NM_000088.3	5′-CTGGAAGAGTGGAGAGTACTG	5′-GTCTCCATGTTGCAGAAGAC-3′
CA2	NM_000067.2	5′-AACAATGGTCATGCTTTCAACG-3′	5′-TGTCCATCAAGTGAACCCCAG-3′
RPL38	NM_000999.3	5′-TCAAGGACTTCCTGCTCACA-3′	5′-AAAGGTATCTGCTGCATCGAA-3′

The above cell experiments were completed in the cooperative laboratory.

2.3 Measurement of the Radical Protection Factor (RPF)

The RPF technology permits to define the antioxidant capacity of samples by measuring the amount of labeled radicals scavenged [38]. In our study, we used electron paramagnetic resonance (EPR) spectroscopy (X-band EPR spectrometer (9.4 GHz) MiniScope MS5000, Magnettech, Freiberg Instruments, Freiberg, Germany) in the following settings: modulation amplitude 2 G, microwave (MW) attenuation 15 dB (MW power 3.16 mW), sweep time 20 s, sweep 95 G, B0-field 3350 G, step 1024 and number(pass) 1 to measure the RPF of two creams.

The labeled test radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, Steinheim, Germany) determines signal intensity of the sample, and the signal intensity is positively correlated with the DPPH concentration, that is, the stronger the signal, the higher the labeled test radical concentration, indicating that the free radical scavenging ability of the sample is lower.

Based on the above theory, first we prepared a test radical DPPH solution, and recorded the concentration of the DPPH solution as RC in radicals/ml. Next, the two creams were diluted in ethanol. In this study, 50 mg of each cream was diluted in 10 ml of ethanol, and an equal amount of this solution was mixed with the prepared DPPH solution (400 µl each), after which we got the amount of this product input (PI) in mg/ml. The first measurement of DPPH signal intensity was started immediately after mixing. Then samples were continuously agitated in a controlled environment (light-proof, room temperature). We repeated the above steps once an hour until the signal intensity was

stable (after about 19 hours). The DPPH signal intensity of the sample treated with creams (verum cream and placebo cream) was the stable signal intensity value and was recorded as “EPR signal sample”. At the same time, an equal amount of DPPH solution was measured under the same condition and the same measurement method described above to obtain the DPPH signal intensity of the control group, which was recorded as “EPR signal control”.

The reduction factor (RF) refers to the ratio of the difference between the “EPR signal control” and the “EPR signal sample” to the “EPR signal control”.

Finally, the RPF of the test sample was calculated by the following formula, and the unit is: 10^{14} radicals/mg.

$$RPF = \frac{RC \cdot RF}{PI}$$

2.4 Ex vivo investigations of penetration depth

2.4.1. Preparations of porcine ear skin

We used fresh pig ears (9 in total) provided by a local slaughterhouse for the penetration depth investigations.

The pig ears were prepared as follows: Without damaging the stratum corneum, they were first cleaned using running water at room temperature, gently dried with paper tissues, and then long hairs on the porcine skin were cut off. Five areas (1 cm · 2 cm)were labled on the pig's ear skin with a marker. Two doses (1 mg/cm^2 and 2 mg/cm^2) of two creams (verum cream and placebo cream) were then applied on the marked areas, while a blank area was left as a control group. The treated pig ears were then placed at 32°C and 90% humidity for 2 hours of passive penetration. We used the same method to mark and treat the other 5 areas from the same pig ear as described above,while they were placed at the same condition for 4 hours of passive penetration.

Finally, more than 8 test points free of hair and furrows were assessed of each marked area by confocal Raman micro spectroscopy (CRM).

2.4.2. Confocal Raman microspectroscopy

The skin penetration depth of both creams was measured using a confocal Raman microscope (Model 3510 SCA, RiverD International B.V., Rotterdam, The Netherlands) with

an excitation wavelength of 785 nm. The measurement of Raman spectra started from the skin surface and was recorded at 2 μm increments down to a depth of 40 μm. The Raman spectra were recorded at the fingerprint region (400–2000 cm^{-1}), the acquisition time of each spectrum was 5 s and the maximal laser power on the skin was 20 mW.

After measuring the Raman spectra, the concentration semiquantitative penetration curves of the two creams were assessed using the 'Skin Tools 2.0' software developed by RiverD International B.V based on the method described by ChunSik Choe et al. [39,40].

Then, using the method described in the paper by Lohan, S.B.,Darvin, M.E et al.[41,42], the intersection of cream-treated skin's and non-treated skin's concentration curves (obtained by Skin Tools 2.0) was taken as the penetration depth value of the cream.

2.5 Volunteers and study design

A total of 10 patients with mild to moderate AD was included in this study. The severity of AD was determined according to the SCORAD scale. Patients with a SCORAD scale less than 50 were eligible to participate as volunteers in the study [43]. Approval for the clinical trial was obtained from the ethics committee of the Charité – Universitätsmedizin Berlin (EA1/207/20). The study was conducted according to the Declaration of Helsinki as revised in 2013, and informed consent was obtained from all patients. Key exclusion criteria included: (i) AD patients with severe infections that require topical or systemic antibiotics therapy; (ii) AD patients with other dermatologic diagnoses; (iii) AD patients who cannot take their independent responsibility; (iv) patients with AD who are pregnant or lactating;(v) AD patients younger than 18 or older than 60.

The study period was 4 weeks. First, bilateral symmetrical parts of AD patients with skin lesions were selected as the test area, and the initial values of each evaluation item on both sides were recorded. Then two creams, respectively, were applied on both sides (placebo and verum were randomly assigned), twice a day (morning and evening) by the patients themselves. Last, the test area was reassessed once the four weekly application time of the two creams had ended.

Placebo and verum were coded by a pharmacist (A or B) to establish a double-blind study.

2.6 Clinical assessment

2.6.1. Assessment of the local SCORAD

Given that this is a half-side, placebo-controlled clinical study of a new topical formulation, the local SCORAD (range from 0 -18) [44] was taken to assess the severity of lesions, with the specific parameters shown in Table 4. The most representative skin lesion was selected from the test area of both sides, such as the bilateral forearm, as the area for evaluating the local SCORAD on this side. Then the testers gave individual scores from 0 to 3 according to the severity of the 6 evaluation items of the representative skin lesion, and the final local SCORAD was determined by the sum of the scores of the above 6 individual items. All the assessments were done by the same person.

Table 4. Scoring criteria for local SCORAD in AD patients (adapted from Yu Zhang et al., 2022[1]).

none(0),mild (1),moderate(2),severe (3)	Intensity[right]	Intensity[left]	Intensity[right]	Intensity[left]
	baseline	baseline	4 weeks later	4 weeks later
erythema				
edema/papulation				
oozing/crusts				
excoriation				
lichenification				
dryness				
Total intensity				

2.6.2. Assessment of itch/sleeplessness

A visual analogue scale (VAS) from 0 to 10 was used to evaluate the intensity of itch/sleeplessness in AD patients [44], in which 0 stands for no perceptible itching/sleeplessness, and 10 stands for worst imaginable itch/sleeplessness.

2.6.3. Assessment of transepidermal water loss (TEWL), stratum corneum hydration (capacitance) and erythema

TEWL, capacitance and erythema of the skin lesions were measured by Tewameter TM 300, the Corneometer CM825 and the Mexameter MX 18 (Courage & Khazaka Electronic GmbH, Cologne, Germany) respectively. Volunteers did not use any cream on the test area 24 hours before the measurement. Before starting the measurement, the volunteers rested for more than 15 minutes in a controlled environment with a room temperature of 20-25°C and a relative humidity of 40%-60% for acclimatation [38,39].

Finally, the average value of three stable continuous test values of skin capacitance and erythema, and the average value of 20 continuous measurement values of TEWL were recorded as the measured value.

2.7 Data analysis

GraphPad Prism (8) was applied for in vitro cellular experiments.Normality was tested by D'Agostino & Pearson omnibus normality test. For data deviating from normality, the non-parametric Kruskal-Wallis test with subsequent Dunn's Multiple Comparison test was used to determine significant differences.

Data for which normality was established, although with different variances, significant differences were documented by a Welch's-ANOVA with subsequent Dunett´s T3 Multiple comparison test. p-values were marked by asterisks: * $p < 0.05$, ** $p < 0.01$.

In vivo investigation intra-group comparisons were obtained by the Wilcoxon signed ranks test, while inter-group comparisons were performed using the Mann–Whitney U-test. SPSS 25 was applied for calculations. Differences with p-values of less than 0.05 were considered statistically significant.

3. Results

3.1 Positive effects of the extract in AD-like in vitro model

The expression of barrier protein molecules and extracellular matrix protein COL1A1 was significantly lower in the in vitro model of AD compared to the normal skin model (untreated keratinocytes) (Figure 1). Although the expression of involucrin was not statistically different in the two models ($p = 0.069$), it showed a significant downward trend in the AD model (Figure 1c). In addition to changes in barrier protein molecules, the strong expression of IL-24, CA2 and CCL26 in the AD model reflected an AD-type inflammatory response formed by cytokine stimulation (Figure 2). Therefore, it could be demonstrated that this AD model can mimic the skin barrier dysfunction and inflammatory response in AD.

The addition of the extract to the AD model significantly upregulated the expression of epidermal differentiation molecules (filaggrin, loricrin and involucrin) and COL1A1 (Figure 1), which exceeded the expression levels of the normal skin model.

The extract also down-regulated the expression of relevant inflammatory factors (Figure 2) to at least normal skin model level (Figure 2b&c), where the expression level of IL-24 was even lower than that in the normal skin model (Figure 2a).

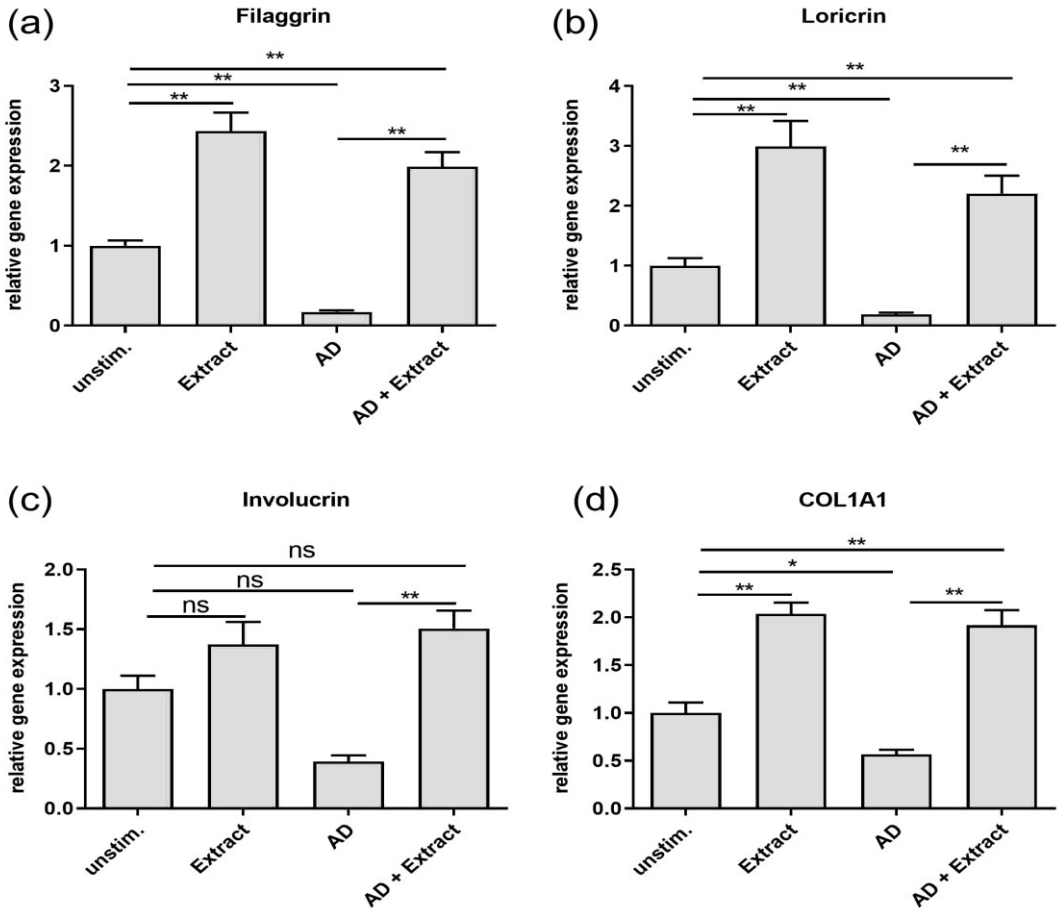


*Figure 1. The extract's effect on the gene expression of skin barrier molecules and extracellular matrix molecules in the in vitro AD-like model. NHEKs-unstim were NHEKs cells without treatment which reflect healthy skin conditions. NHEKs treated with an AD cytokine mix (IL-22, TNF-α, IL-13 and IL-4 (10 ng/mL each)) reflect AD skin conditions ,which were mixed either in blank (AD) or in the extract (AD + Extract). Gene expression levels of (a) filaggrin (b) loricrin (c) involucrin (d) COL1A1 were determined by real-time PCR and normalized to the gene expression of the housekeeping gene RP38. p-values were marked as: * p < 0.05, ** p < 0.01 (n = 9–18 stimulations).ns: no significant differences* (*from Yu Zhang et al., 2022[1]).*

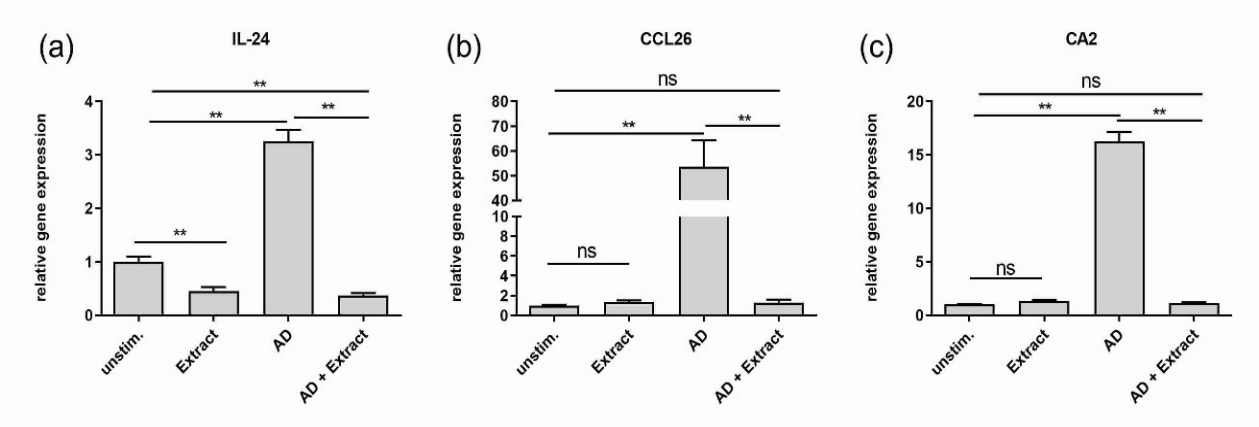


*Figure 2. The extract's effect on the gene expression of inflammation marker in the in vitro AD-like model. NHEKs-unstim were NHEKs cells without treatment which reflect healthy skin conditions. NHEKs treated with an AD cytokine mix (IL-22, TNF-α, IL-13 and IL-4 (10 ng/mL each)) reflect AD skin conditions ,which were mixed either in blank (AD) or in the extract (AD + Extract). Gene expression levels of (a) IL-24 (b) CA2 (c) CCL26 were determined by real-time PCR and normalized to the gene expression of the housekeeping gene RP38. p-values were marked as: ** p < 0.01 (n = 9–18 stimulations). ns: no significant differences (from Yu Zhang et al., 2022[1]).*

3.2 Radical scavenging activity of the formulations

The RPF value of two creams measured by EPR spectroscopy are shown in Table 5.

Table 5. The RPF value of verum cream and placebo cream (from Yu Zhang et al., 2022[1]).

	Verum cream	Placebo cream
RPF (radicals/mg)	(690 ± 30) × 10^{14}	0

3.3 Penetration studies

Although the strong antioxidant and anti-inflammatory properties, the skin barrier repair function of the extract was confirmed in ex vivo experiments, a CRM system was required to determine whether or not these functional components could penetrate the stratum corneum and reach the active layer of the epidermis.

The averaged Raman spectrum of the two creams showed a large difference in fluorescence intensity (Figure 3). The strong fluorescence background was related to the extract, which enabled the comparison between verum cream and placebo cream accomplished by CRM (Figure 4).

The penetration curves of the two creams are shown in Figure 5. The concentration of penetrated compounds of both creams peaked at the skin surface and then decreased exponentially. At a skin depth of 20-25 µm, the fluorescence intensity of both creams was consistent with that of the control group (untreated skin) for both penetration times (2 hours and 4 hours). Since the thickness of the stratum corneum is usually about 18 µm [40], it can be inferred that some of the contents of both creams and especially the extract can penetrate the stratum corneum and thus reach the active epidermis. Nevertheless,

the available data do not demonstrate a significant difference in the penetration depth of the two creams.

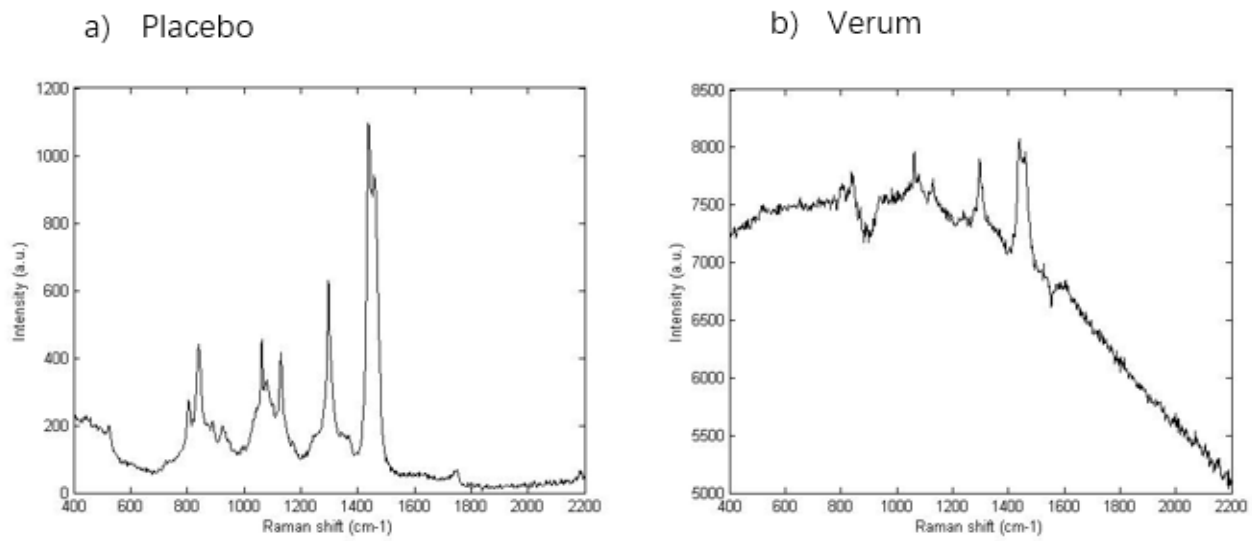
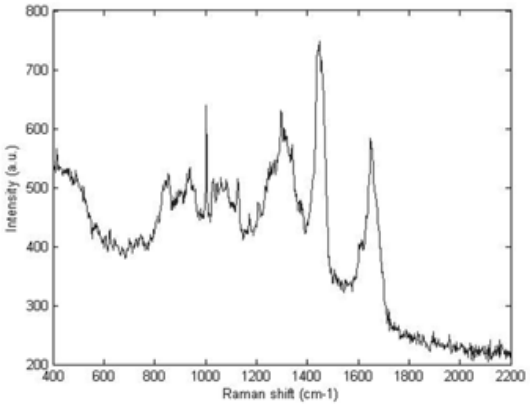


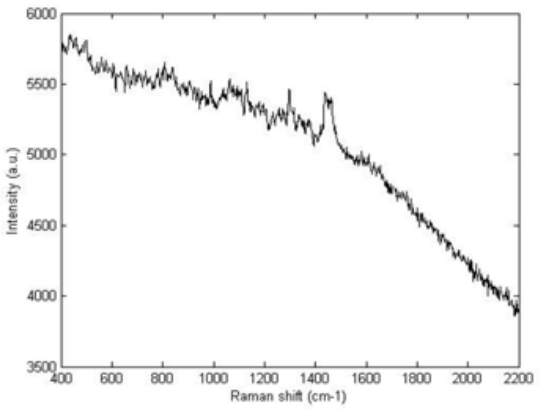
Figure 3. Representative averaged Raman spectrum of verum cream (a) and placebo cream (b) (own figure).

a) placebo-treated skin

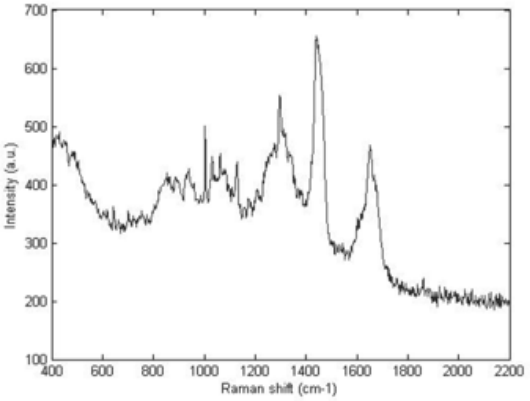
b) verum-treated skin

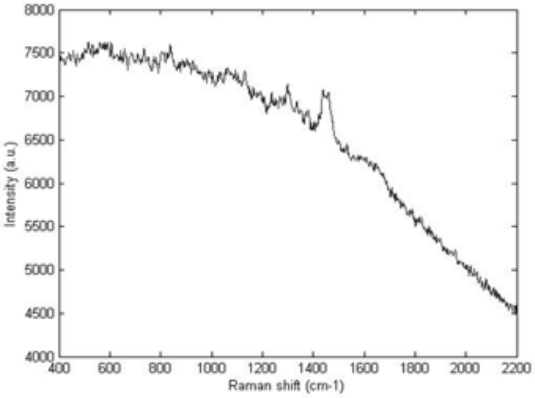
2 µm



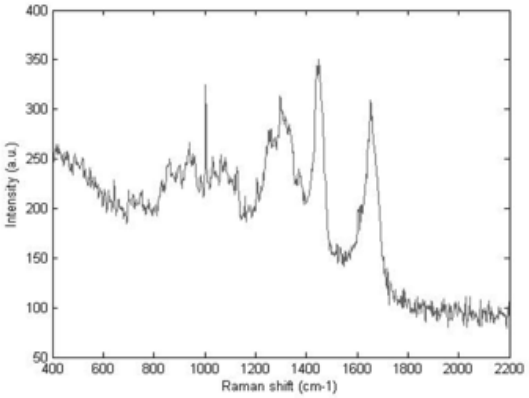


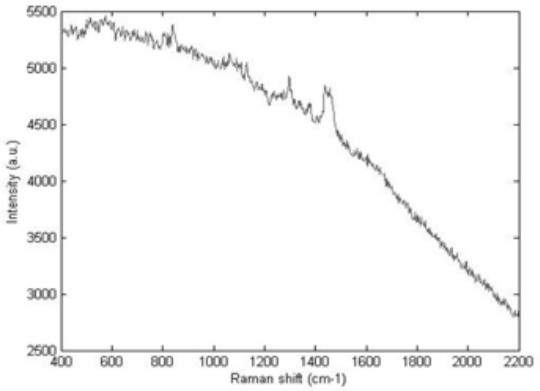
8 µm



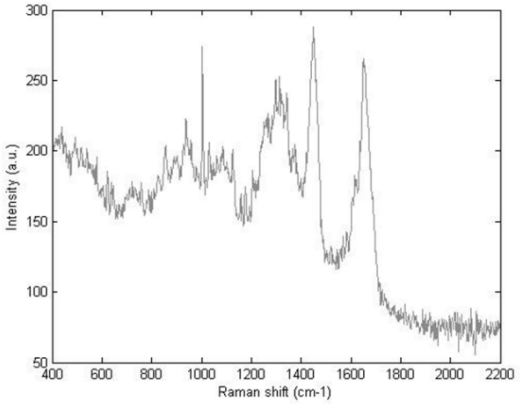


16 µm





22 µm



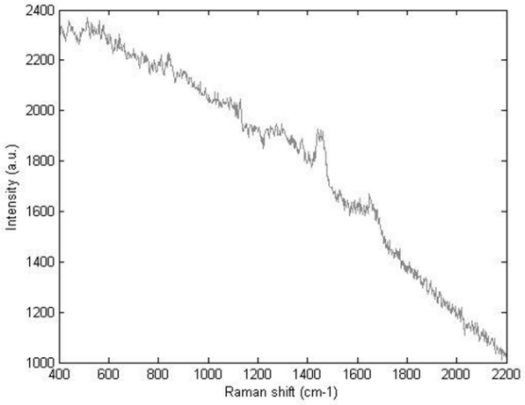


Figure 4. Representative averaged Raman spectrum of placebo cream-treated skin (a) and verum cream-treated skin (b) recorded at different skin depths (2, 8, 16 and 22 µm) (own figure).

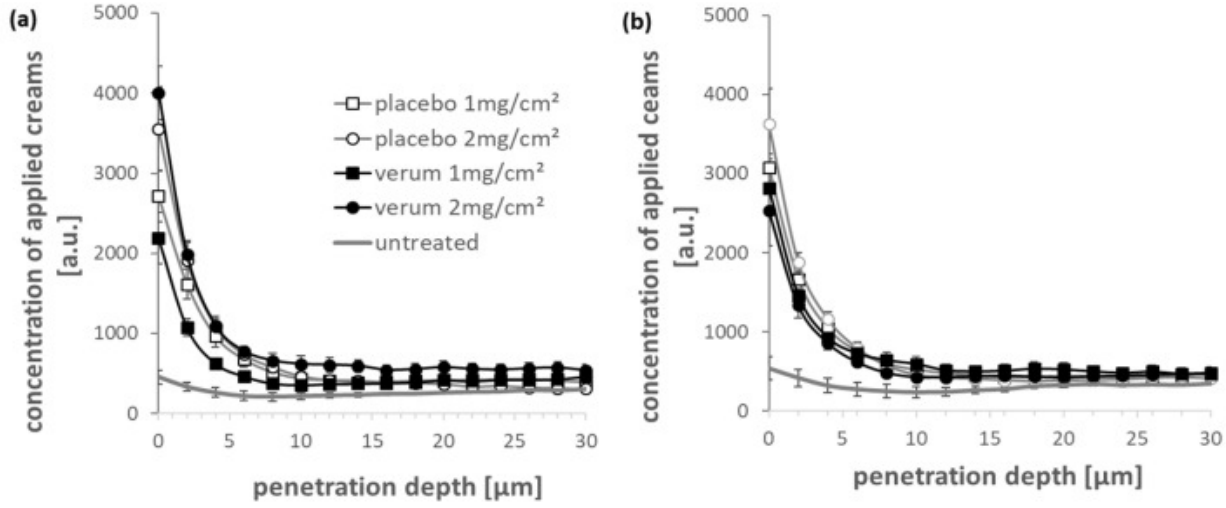


Figure 5. The penetration depth of verum and placebo creams on porcine skin after (a) 2h and (b) 4h penetration time. Untreated skin was used as blank control (*from Yu Zhang et al., 2022[1]).*

3.4 Clinical study

A total of 10 patients with mild to moderate AD was included, and the general information of the patients is shown in Table 6.

Table 6. General information of enrolled AD patients (*from Yu Zhang et al., 2022[1]).*

patient num	1	2	3	4	5	6	7	8	9	10
age/years	27	29	23	37	35	23	35	35	61	25
gender F/M	F	F	M	M	F	F	F	F	M	F
test area	upper arms forearms	hands	hands	forearms	shin	upper arms	forearms	upper arms	forearms	flank abdomen

The absolute values after 4 weeks treatment of all measured items compared to the base values are shown in Table 7.

Among the 6 measured parameters, the local SCORAD, itching and TEWL of skin lesions on verum cream side showed statistical differences before and after treatment, but no significant differences in other 3 measured parameters. In contrast, there were no statistically significant differences in all measured parameters before and after placebo cream treatment (Figure 6). The relative values compared to the baseline (before treatment) are shown in Figure 7.

Table 7. Verum and Placebo creams' effect on clinical and non-invasive bioengineering assessment before (D0)and after 4 weeks' treatment(D28) , Mean ± SEM (n = 10) (*adapted from Yu Zhang et al., 2022[1]).*

	Verum	Placebo
Local SCORAD		
D0	6.3 ± 1.0	5.3 ± 1.0
D28	2.3 ± 0.7	4.1 ± 0.8
Itch		
D0	3.8 ± 0.5	3.9 ± 0.7
D28	1.6 ± 0.4	3.4 ± 0.8
Sleeplessness		
D0	2.9 ± 0.9	2.9 ± 0.9
D28	2.2 ± 0.8	2.2 ± 0.8
TEWL		
D0	27 ± 7	27 ± 8
D28	16 ± 4	25 ± 7
Skin capacitance		
D0	20 ± 5	23 ± 5
D28	28 ± 5	25 ± 5
Skin erythema		
D0	340 ± 30	340 ± 40
D28	340 ± 40	310 ± 20

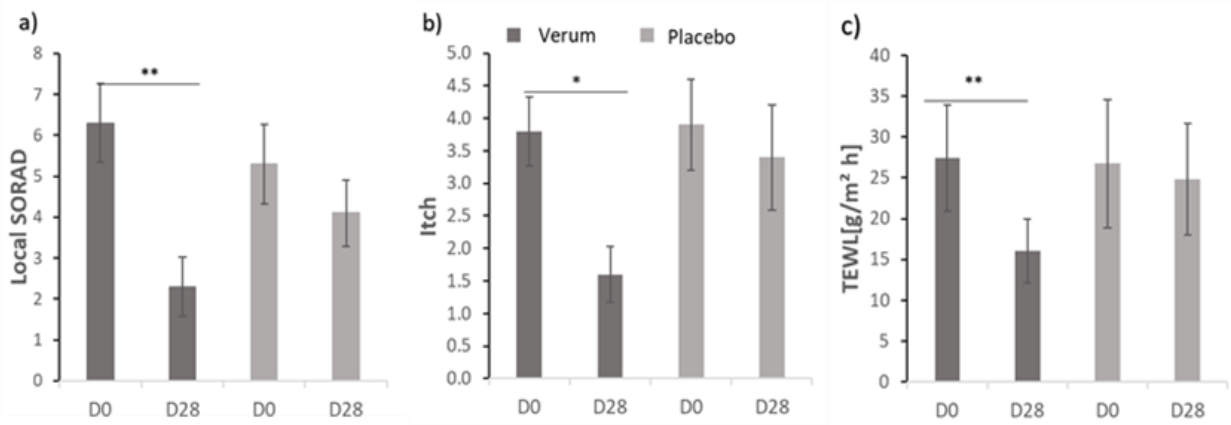


*Figure 6. Verum and Placebo creams' effect comparison of (a) local SCORAD, (b) itch and (c) TEWL before (D0)and after 4 weeks' treatment(D28), Mean ± SEM (n = 10); level of significance * p < 0.05, ** p < 0.01 (from Yu Zhang et al., 2022[1]).*

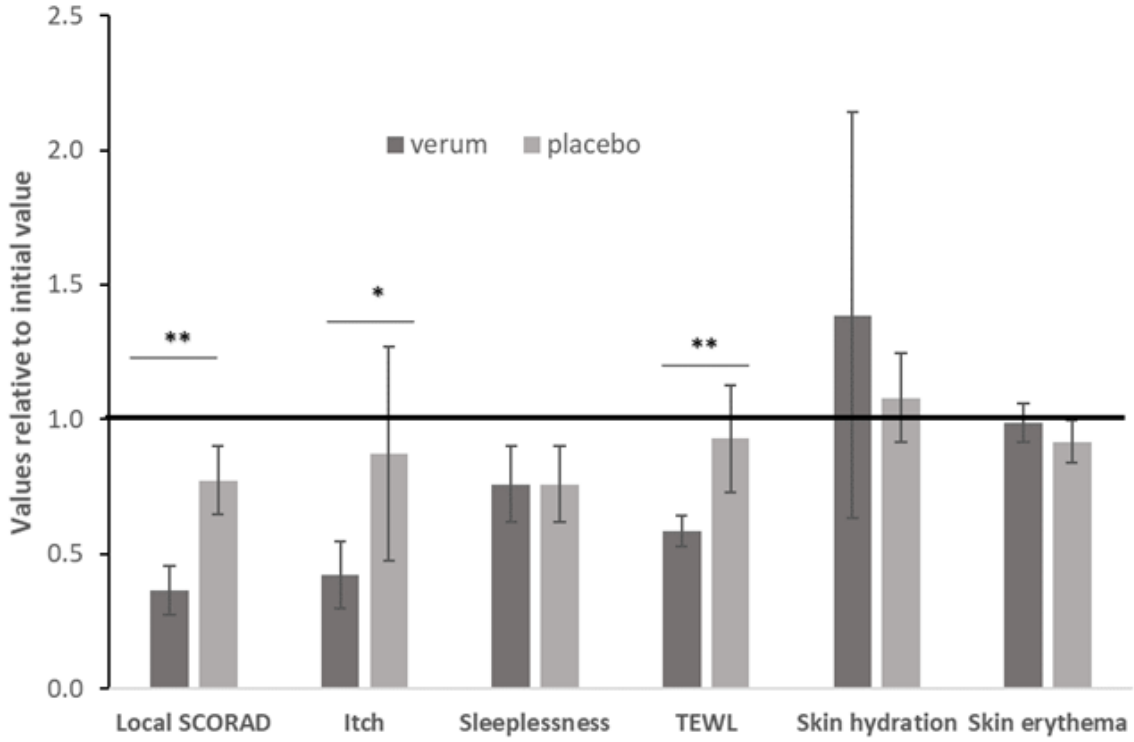


*Figure 7. Comparison of the effects of verum and placebo creams for all outcomes, assessed as relative to baseline; level of significance * p < 0.05, ** p < 0.01(from Yu Zhang et al., 2022[1]).*

Before the creams were applied, there was no significant difference in the severity of skin lesions between the symmetrical test areas, as demonstrated by no statistical difference on local SCORAD, itching intensity and TEWL of skin lesions on both sides at baseline.

After 4 weeks' treatment on the side using verum cream, local SCORAD decreased by more than 50% compared to pre-treatment, the difference was statistically significant (p = 0.005, Figure 6), while the control group dropped by only 22.6% compared with baseline.

The VAS of itch and TEWL values inferred that verum cream could significantly reduce VAS (itch) values (p = 0.011, Figure 6) as well as TEWL values (p = 0.005, Figure 6), although VAS and TEWL values also decreased slightly after treatment in the placebo group, but there was no statistical difference before and after treatment.

Both creams did not have a positive effect on the sleep quality of AD patients and it was not possible to compare the difference in sleep quality improvement between the two creams due to the systematic way in which sleep quality was assessed (Figure 7). There

was no significant difference in the erythema of both groups before and after treatment (Figure 7), similarly, there was also no statistically significant difference in skin capacitance between the two sides before and after treatment. However, a trend toward increased skin capacitance in the treatment group could be seen from the absolute and relative values before and after the 4 weeks' treatment (Figure 7 and Table 7). It can be seen in Figure 7 that the relative values of TEWL, itch, and local SCORAD after application of the verum cream were significantly reduced compared to the values after using placebo cream.

In summary, verum cream could significantly reduce local SCORAD, VAS (itch), and TEWL while the control group could not. Neither cream was effective in reducing erythema, improving sleep quality, nor increasing skin capacitance.

4. Discussion

In recent years, several studies have reported an increasing trend in AD prevalence worldwide [45]. Intensive clinical manifestations and skin barrier damage produce many negative effects on the quality of life and the treatment burden [46]. Therefore, safe and effective treatment strategies are of great importance for patients with AD. The unpredictable and highly heterogeneous causative factors of recurrent acute inflammatory phases and the long-standing chronic inflammation set the basic treatment strategy for AD, that is, a patient-oriented long-term management strategy [47,48]. Anti-inflammatory treatment according to the inflammation degree complemented by the use of moisturizers to restore the skin barrier is the classical principle of AD treatment [47]. For most patients with mild to moderate AD, topical treatment is sufficient to treat the lesions and provide effective clinical relief, while systemic treatment is only considered for severe AD or moderate AD when topical treatment is inadequate [20]. Therefore, corticosteroids and calcineurin inhibitors are often used for topical anti-inflammatory therapy [49]. Although appropriate intermittent use can avoid the risk of skin atrophy, capillary dilation, etc. [49], for some AD patients, such as those with more facial lesions, younger children, and short intervals between recurrence, a topical anti-inflammatory formulation with long-term safe properties and skin barrier enhancement function is necessary.

When the overall burden of oxidation on the reducing agent, favors the oxidizing agent, this state is called oxidative stress. The hallmark pathology of AD is chronic skin inflammation, when in an inflammatory-activated state, the production of oxidants and free radicals increases, subsequently, excess free radicals upregulate the expression of pro-inflammatory cytokines, further exacerbating the inflammatory response [50]. On the one hand, an inflammatory state interacts with the overproduction of oxidants and free radicals, on the other hand, relevant studies have shown the presence of antioxidant system disorders in AD patients [51], which negatively affect disease progression [52,53]. Given the relevance of oxidative stress on AD, the beneficial effects of applying antioxidants to treat AD are gradually gaining attention. Topical application of antioxidants is commonly used to reduce the negative effects caused by UV radiation. Thus, clinical studies on the topical application of antioxidants for the treatment of inflammatory diseases such as AD are rare. Herbal, fruit and vegetable extracts are often added to topical therapeutic formulations [54-56]. Apple, kale and green tea contain dietary antioxidants, with the green tea polyphenols in green tea not only being antioxidants but also having anti-inflammatory

properties [57]. Similarly, studies have shown that applephenon in apples reduces allergic reactions [58,59], and kaempferol in kale reduces oxidative stress, protects dermal collagen, and promotes wound healing [60-64].However, clinical studies on the above extracts have focused on the intake effects instead of topical application. Compared to ingestion, topical application allows higher tissue concentrations and reduces potential risks to other organs [65]

L-arginine, a component of filaggrin, can be converted into NMF [66]. L-arginine can be hydrolyzed to urea and ornithine when catalyzed by arginase I. Both of them can increase the amount of inhibitory peptides and enhance stratum corneum hydration [35,67]. It could produce NO when catalyzed by NO synthase, which has a positive effect on regulating the inflammatory response [68]. Therefore, L-arginine has a positive effect on skin barrier repair.

In recent years, more and more antioxidant studies have been conducted on possible combinations of different antioxidants as opposed to previous studies that applied a single antioxidant. Therefore, in this study, a mixture of extracts from three different sources was used to achieve better therapeutic effects.

Considering the safety and allergenic risk for long-term use, the extracts used in this study are from natural food and do not contain common plant allergens (terpenoids, etc.) [69].

Based on the above theory, we prepared the extract and measured the actual antioxidant and anti-inflammatory capacity of this new extract in the following in vitro experiments.

In order to quantify the antioxidant capacity of this extract, we measured its RPF value using EPR, which was much higher than that of most creams, and even creams with lower RPF values still had high free radical scavenging activity [38,70]. Therefore, it is inferred that this extract has strong antioxidant and free radical scavenging ability. Free radicals can cause damage to some cellular components, eventually leading to a damaged cell, finally disease state or cell death. Endogenous sources of free radicals include inflammatory processes. There are studies that demonstrate the positive effects of topical application of antioxidant formulations with free radical scavenging activity on healthy skin [71,72]. Although the RPF value suggested the antioxidant effect of the extract, further experiments are needed to compare the changes in free radicals within the skin lesions before and after treatment.

In vitro experimental results revealed the anti-inflammatory effect and skin barrier enhancement of the extract. The extracts significantly reduced the expression of IL-24 in

the AD model, which affects the normal barrier function of the skin by down-regulating the expression of filaggrin in keratinocytes [73]. A significant decrease in CCL26, a chemokine produced by IL-4 and IL-13, was also seen in the AD model, demonstrating the inhibitory effect of the extract on the inflammatory process dominated by the TH2 immune response.CA2 is commonly expressed in various tissues including skin. It is a key enzyme in maintaining cellular pH. The pH of the normal skin surface is 3-5, the lower pH prevents the colonization of some harmful bacteria. The strong expression of CA2 could be seen in AD lesions, which speculated that it may contribute to the increased pH of skin lesions in AD patients [74,75], thus harmful bacteria colonize more easily and further disrupt the damaged skin barrier. This extract mixture was effective in reducing the induction of CA2 in AD models. As mentioned earlier, filaggrin, involucrin and loricrin are important proteins for the skin barrier function, and their expression decreases due to skin barrier dysfunction, while this extract mixture can significantly increase their expression and thus enhance the damaged skin barrier.

Given the chemical properties of antioxidants, the penetration depth of antioxidant-rich formulations cannot be ignored. If the antioxidant remains only on the surface of the skin, it may oxidize more easily, thus failing to protect the cells in the lower layers from free radical damage. Then its antioxidant capacity can be misleading. We therefore measured the penetration depth of both creams ex vivo on pig ear skin by CRM. As can be seen in Figure 4, most of the ingredients stayed on skin surface and stratum corneum, but some components from both creams could penetrate the stratum corneum and reached the active layer of epidermis. The penetration depth of both creams was essentially the same, around 20 µm, and did not correlate significantly with the penetration time. In this study, we did not measure the penetration depths of individual components of the extract because the fluorescence of the extract was still visible at 20 µm below the stratum corneum (Figure 3). The spectra of the creams illustrated that the fluorescence is related to the extract mixture, so we can conclude that the extract mixture reached the viable epidermis which explains the in vivo results. Furthermore, cream components penetrated into the stratum corneum could be seen as a ‘storage’ which could provide sufficient penetration time for active substances such as antioxidants into the active cellular layer.

In vitro and ex vivo data provided the possibility of using the extract mixture in topical treatment of AD, while a 4-week randomized, double-blind, semi-lateral comparative clinical study revealed the efficiency of the extract mixture on the improvement of clinical symptoms and skin lesions in AD patients. Patients did not use any therapeutic creams

or skin care products during the study, while the half-sided comparative design excluded the effect of individual heterogeneity on the results. The local SCORAD is the most intuitive scoring system for the severity of lesions on AD patients. It includes both skin changes during the acute inflammatory phase (erythema, oozing, etc.) and those resulting from chronic inflammation (lichenification). In the clinical trial, the local SCORAD was significantly reduced in 10 patients of the verum group, and in even 3 of them the lesions disappeared completely after the use of the verum cream; while the local SCORAD on the control side remained essentially unchanged or decreased only slightly after treatment. Pruritus is the most common and most difficult clinical symptom of AD to remove. It affects the patient's life and psychological health [76], while scratching can disrupt the skin barrier and lead to bacterial colonization and inflammatory reactions. Skin barrier dysfunction in AD patients leads to the expression of various pro-inflammatory mediators (e.g. neuropeptides) and the penetration of irritants and pruritic substances, resulting in pruritus symptoms [77]. For most of the 10 verum patients, the cream containing the extracts significantly improved their pruritic symptoms or they even disappeared, while the control cream barely improved the pruritic symptoms or even exacerbated the itchiness of the lesions. The skin is an organ with a barrier function, on the one hand it blocks exogenous substances and on the other hand it prevents transepidermal water loss. The Tewameter probe allows non-invasive measurement of the skin's TEWL and thus assessment of the skin's barrier function [78]. Skin lesions treated with verum cream exhibited a significant decline in TEWL, again substantiating the positive benefits of the extracts on skin barrier. The hydration of the stratum corneum is a reflection of the degree of dryness of the skin [79]. The water content of the stratum corneum is related to the following 2 factors: the water molecules bonding ability of the NMF in corneocytes and the hydrophilic part in lipid lamellas [80-82]; the structure formed by corneocytes and lipid matrix, which could regulate TEWL [83,84]. Thus, skin hydration, i.e., skin capacitance, can reflect the skin barrier function to some extent. Although the clinical data could not prove a difference before and after verum cream treatment, the relative values of the verum and placebo-treated areas showed a trend towards elevated skin capacitance for the verum cream. It is speculated that this may be related to the small sample size.

Skin erythema is the dilatation of skin capillaries caused by infections, allergies, etc. In AD patients, erythema is usually caused by a persistent inflammatory response. Both creams failed to improve erythema, given that the new cream, although having some anti-inflammatory effect, has a weaker anti-inflammatory effect than topical steroids and does

not relieve capillary dilation caused by inflammation. No adverse events occurred during the clinical trial and the cream was well tolerated by patients, but further follow-up is needed to determine the safety of its long-term use. Also, the small sample size restricts the comparison between this topical formulation and commonly used first-line topical treatments. In addition, the new cream has a strong smell and poor flowability, which should be improved. In conclusion, the anti-inflammatory, antioxidant and skin barrier repair properties of the selected extracts were confirmed by the results of the in vitro AD-model, meanwhile the results of penetration studies provide the basis for the extract mixture to exert its therapeutic effects in the epidermis. The 4-week clinical study provided effective evidence for the use of this extract mixture in AD treatment. The aims of the study were achieved: A new safe formulation has been provided for the topical treatment of AD patients.

While the results of this study are generally consistent with the published literature, its design was focused for the first time on the investigation of the therapeutic effects in vitro of a mixture of natural extracts from different sources, the epidermal penetration efficiency of this mixture, and the treatment efficiency of singularly using a non-steroid or immunomodulator topical cream on mild to moderate AD patients.

5. Conclusions

The results of the in vitro cellular assay indicated the anti-inflammatory effect and the skin barrier repair function of the extract mixture, while the RPF values measured by EPR determined its strong antioxidant capacity, the above researches provide a theoretical basis for the use of cream enriched with the extract in AD treatment. The penetration curve measured by CRM showed the possibility of the formulation penetrating the stratum corneum to reach the active layer of the skin to exert antioxidant effects. In the in vivo study, the improvement of local lesions and the relief of clinical symptoms such as pruritus further confirmed the above findings, which may provide a new skin care product for the treatment of AD patients.

Reference list

1. Zhang, Y.; Heinemann, N.; Rademacher, F.; Darvin, M.E.; Raab, C.; Keck, C.M.; Vollert, H.; Fluhr, J.W.; Gläser, R.; Harder, J.; Meinke, M.C. Skin Care Product Rich in Antioxidants and Anti-Inflammatory Natural Compounds Reduces Itching and Inflammation in the Skin of Atopic Dermatitis Patients. *Antioxidants (Basel, Switzerland)* **2022**, *11*, doi:10.3390/antiox11061071.
2. Eichenfield, L.F.; Tom, W.L.; Berger, T.G.; Krol, A.; Paller, A.S.; Schwarzenberger, K.; Bergman, J.N.; Chamlin, S.L.; Cohen, D.E.; Cooper, K.D.; Cordoro, K.M.; Davis, D.M.; Feldman, S.R.; Hanifin, J.M.; Margolis, D.J.; Silverman, R.A.; Simpson, E.L.; Williams, H.C.; Elmets, C.A.; Block, J.; Harrod, C.G.; Smith Begolka, W.; Sidbury, R. Guidelines of care for the management of atopic dermatitis: section 2. Management and treatment of atopic dermatitis with topical therapies. *Journal of the American Academy of Dermatology* **2014**, *71*, 116-132, doi:10.1016/j.jaad.2014.03.023.
3. van Zuuren, E.J.; Fedorowicz, Z.; Christensen, R.; Lavrijsen, A.; Arents, B.W.M. Emollients and moisturisers for eczema. *The Cochrane database of systematic reviews* **2017**, *2*, Cd012119, doi:10.1002/14651858.CD012119.pub2.
4. Miller, J.J.; Roling, D.; Margolis, D.; Guzzo, C. Failure to demonstrate therapeutic tachyphylaxis to topically applied steroids in patients with psoriasis. *Journal of the American Academy of Dermatology* **1999**, *41*, 546-549.
5. Teo, C.W.L.; Tay, S.H.Y.; Tey, H.L.; Ung, Y.W.; Yap, W.N. Vitamin E in Atopic Dermatitis: From Preclinical to Clinical Studies. *Dermatology (Basel, Switzerland)* **2021**, *237*, 553-564, doi:10.1159/000510653.
6. Arulselvan, P.; Fard, M.T.; Tan, W.S.; Gothai, S.; Fakurazi, S.; Norhaizan, M.E.; Kumar, S.S. Role of Antioxidants and Natural Products in Inflammation. *Oxidative medicine and cellular longevity* **2016**, *2016*, 5276130, doi:10.1155/2016/5276130.
7. Weidinger, S.; Novak, N. Atopic dermatitis. *Lancet (London, England)* **2016**, *387*, 1109-1122, doi:10.1016/s0140-6736(15)00149-x.
8. Sacotte, R.; Silverberg, J.I. Epidemiology of adult atopic dermatitis. *Clinics in dermatology* **2018**, *36*, 595-605, doi:10.1016/j.clindermatol.2018.05.007.
9. Kulthanan, K.; Samutrapong, P.; Jiamton, S.; Tuchinda, P. Adult-onset atopic dermatitis: a cross-sectional study of natural history and clinical manifestation. *Asian Pacific journal of allergy and immunology* **2007**, *25*, 207-214.
10. Furue, M. Regulation of Filaggrin, Loricrin, and Involucrin by IL-4, IL-13, IL-17A, IL-22, AHR, and NRF2: Pathogenic Implications in Atopic Dermatitis. *International journal of molecular sciences* **2020**, *21*, doi:10.3390/ijms21155382.
11. Oiso, N.; Fukai, K.; Ishii, M. Interleukin 4 receptor alpha chain polymorphism Gln551Arg is associated with adult atopic dermatitis in Japan. *The British journal of dermatology* **2000**, *142*, 1003-1006, doi:10.1046/j.1365-2133.2000.03485.x.
12. Heine, G.; Hoefer, N.; Franke, A.; Nöthling, U.; Schumann, R.R.; Hamann, L.; Worm, M. Association of vitamin D receptor gene polymorphisms with severe atopic dermatitis in adults. *The British journal of dermatology* **2013**, *168*, 855-858, doi:10.1111/bjd.12077.
13. Clark, A.; Mach, N. Role of Vitamin D in the Hygiene Hypothesis: The Interplay between Vitamin D, Vitamin D Receptors, Gut Microbiota, and Immune Response. *Frontiers in immunology* **2016**, *7*, 627, doi:10.3389/fimmu.2016.00627.
14. Kezic, S.; Kemperman, P.M.; Koster, E.S.; de Jongh, C.M.; Thio, H.B.; Campbell, L.E.; Irvine, A.D.; McLean, W.H.; Puppels, G.J.; Caspers, P.J. Loss-of-function

mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum. *The Journal of investigative dermatology* **2008**, *128*, 2117-2119, doi:10.1038/jid.2008.29.
15. O'Regan, G.M.; Sandilands, A.; McLean, W.H.; Irvine, A.D. Filaggrin in atopic dermatitis. *The Journal of allergy and clinical immunology* **2009**, *124*, R2-6, doi:10.1016/j.jaci.2009.07.013.
16. Munera-Campos, M.; Carrascosa, J.M. Innovation in Atopic Dermatitis: From Pathogenesis to Treatment. *Actas dermo-sifiliograficas* **2020**, *111*, 205-221, doi:10.1016/j.ad.2019.11.002.
17. Renert-Yuval, Y.; Guttman-Yassky, E. New treatments for atopic dermatitis targeting beyond IL-4/IL-13 cytokines. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology* **2020**, *124*, 28-35, doi:10.1016/j.anai.2019.10.005.
18. Furue, M.; Ulzii, D.; Vu, Y.H.; Tsuji, G.; Kido-Nakahara, M.; Nakahara, T. Pathogenesis of Atopic Dermatitis: Current Paradigm. *Iranian journal of immunology : IJI* **2019**, *16*, 97-107, doi:10.22034/iji.2019.80253.
19. Langan, S.M.; Irvine, A.D.; Weidinger, S. Atopic dermatitis. *Lancet (London, England)* **2020**, *396*, 345-360, doi:10.1016/s0140-6736(20)31286-1.
20. Simpson, E.L.; Bruin-Weller, M.; Flohr, C.; Ardern-Jones, M.R.; Barbarot, S.; Deleuran, M.; Bieber, T.; Vestergaard, C.; Brown, S.J.; Cork, M.J.; Drucker, A.M.; Eichenfield, L.F.; Foelster-Holst, R.; Guttman-Yassky, E.; Nosbaum, A.; Reynolds, N.J.; Silverberg, J.I.; Schmitt, J.; Seyger, M.M.B.; Spuls, P.I.; Stalder, J.F.; Su, J.C.; Takaoka, R.; Traidl-Hoffmann, C.; Thyssen, J.P.; van der Schaft, J.; Wollenberg, A.; Irvine, A.D.; Paller, A.S. When does atopic dermatitis warrant systemic therapy? Recommendations from an expert panel of the International Eczema Council. *Journal of the American Academy of Dermatology* **2017**, *77*, 623-633, doi:10.1016/j.jaad.2017.06.042.
21. Wollenberg, A.; Barbarot, S.; Bieber, T.; Christen-Zaech, S.; Deleuran, M.; Fink-Wagner, A.; Gieler, U.; Girolomoni, G.; Lau, S.; Muraro, A.; Czarnecka-Operacz, M.; Schäfer, T.; Schmid-Grendelmeier, P.; Simon, D.; Szalai, Z.; Szepietowski, J.C.; Taïeb, A.; Torrelo, A.; Werfel, T.; Ring, J. Consensus-based European guidelines for treatment of atopic eczema (atopic dermatitis) in adults and children: part I. *Journal of the European Academy of Dermatology and Venereology : JEADV* **2018**, *32*, 657-682, doi:10.1111/jdv.14891.
22. Hengge, U.R.; Ruzicka, T.; Schwartz, R.A.; Cork, M.J. Adverse effects of topical glucocorticosteroids. *Journal of the American Academy of Dermatology* **2006**, *54*, 1-15; quiz 16-18, doi:10.1016/j.jaad.2005.01.010.
23. Charman, C.; Williams, H. The use of corticosteroids and corticosteroid phobia in atopic dermatitis. *Clinics in dermatology* **2003**, *21*, 193-200, doi:10.1016/s0738-081x(02)00368-1.
24. Siegfried, E.C.; Jaworski, J.C.; Hebert, A.A. Topical calcineurin inhibitors and lymphoma risk: evidence update with implications for daily practice. *American journal of clinical dermatology* **2013**, *14*, 163-178, doi:10.1007/s40257-013-0020-1.
25. Bertino, L.; Guarneri, F.; Cannavò, S.P.; Casciaro, M.; Pioggia, G.; Gangemi, S. Oxidative Stress and Atopic Dermatitis. *Antioxidants (Basel, Switzerland)* **2020**, *9*, doi:10.3390/antiox9030196.
26. van den Bogaard, E.H.; Bergboer, J.G.; Vonk-Bergers, M.; van Vlijmen-Willems, I.M.; Hato, S.V.; van der Valk, P.G.; Schröder, J.M.; Joosten, I.; Zeeuwen, P.L.; Schalkwijk, J. Coal tar induces AHR-dependent skin barrier repair in atopic

dermatitis. *The Journal of clinical investigation* **2013**, *123*, 917-927, doi:10.1172/jci65642.
27. Kim, B.E.; Leung, D.Y.; Boguniewicz, M.; Howell, M.D. Loricrin and involucrin expression is down-regulated by Th2 cytokines through STAT-6. *Clinical immunology (Orlando, Fla.)* **2008**, *126*, 332-337, doi:10.1016/j.clim.2007.11.006.
28. Bao, L.; Shi, V.Y.; Chan, L.S. IL-4 regulates chemokine CCL26 in keratinocytes through the Jak1, 2/Stat6 signal transduction pathway: Implication for atopic dermatitis. *Molecular immunology* **2012**, *50*, 91-97, doi:10.1016/j.molimm.2011.12.008.
29. Kagami, S.; Kakinuma, T.; Saeki, H.; Tsunemi, Y.; Fujita, H.; Nakamura, K.; Takekoshi, T.; Kishimoto, M.; Mitsui, H.; Torii, H.; Komine, M.; Asahina, A.; Tamaki, K. Significant elevation of serum levels of eotaxin-3/CCL26, but not of eotaxin-2/CCL24, in patients with atopic dermatitis: serum eotaxin-3/CCL26 levels reflect the disease activity of atopic dermatitis. *Clinical and experimental immunology* **2003**, *134*, 309-313, doi:10.1046/j.1365-2249.2003.02273.x.
30. Zhang, R.; Ai, X.; Duan, Y.; Xue, M.; He, W.; Wang, C.; Xu, T.; Xu, M.; Liu, B.; Li, C.; Wang, Z.; Zhang, R.; Wang, G.; Tian, S.; Liu, H. Kaempferol ameliorates H9N2 swine influenza virus-induced acute lung injury by inactivation of TLR4/MyD88-mediated NF-κB and MAPK signaling pathways. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* **2017**, *89*, 660-672, doi:10.1016/j.biopha.2017.02.081.
31. Liu, C.; Liu, H.; Lu, C.; Deng, J.; Yan, Y.; Chen, H.; Wang, Y.; Liang, C.L.; Wei, J.; Han, L.; Dai, Z. Kaempferol attenuates imiquimod-induced psoriatic skin inflammation in a mouse model. *Clinical and experimental immunology* **2019**, *198*, 403-415, doi:10.1111/cei.13363.
32. 松崎, 妙.; 原, 征. 茶葉カテキン類の抗酸化作用について. *日本農芸化学会誌* **1985**, *59*, 129-134, doi:10.1271/nogeikagaku1924.59.129.
33. Nichols, J.A.; Katiyar, S.K. Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms. *Archives of dermatological research* **2010**, *302*, 71-83, doi:10.1007/s00403-009-1001-3.
34. Zhai, Y.; Dang, Y.; Gao, W.; Zhang, Y.; Xu, P.; Gu, J.; Ye, X. P38 and JNK signal pathways are involved in the regulation of phlorizin against UVB-induced skin damage. *Experimental dermatology* **2015**, *24*, 275-279, doi:10.1111/exd.12642.
35. Grether-Beck, S.; Felsner, I.; Brenden, H.; Kohne, Z.; Majora, M.; Marini, A.; Jaenicke, T.; Rodriguez-Martin, M.; Trullas, C.; Hupe, M.; Elias, P.M.; Krutmann, J. Urea uptake enhances barrier function and antimicrobial defense in humans by regulating epidermal gene expression. *The Journal of investigative dermatology* **2012**, *132*, 1561-1572, doi:10.1038/jid.2012.42.
36. Bernard, F.X.; Morel, F.; Camus, M.; Pedretti, N.; Barrault, C.; Garnier, J.; Lecron, J.C. Keratinocytes under Fire of Proinflammatory Cytokines: Bona Fide Innate Immune Cells Involved in the Physiopathology of Chronic Atopic Dermatitis and Psoriasis. *Journal of allergy* **2012**, *2012*, 718725, doi:10.1155/2012/718725.
37. Roth, S.A.; Simanski, M.; Rademacher, F.; Schröder, L.; Harder, J. The pattern recognition receptor NOD2 mediates Staphylococcus aureus-induced IL-17C expression in keratinocytes. *The Journal of investigative dermatology* **2014**, *134*, 374-380, doi:10.1038/jid.2013.313.
38. Meinke, M.C.; Haag, S.F.; Schanzer, S.; Groth, N.; Gersonde, I.; Lademann, J. Radical protection by sunscreens in the infrared spectral range. *Photochemistry and photobiology* **2011**, *87*, 452-456, doi:10.1111/j.1751-1097.2010.00838.x.

39. Caspers, P.J.; Lucassen, G.W.; Carter, E.A.; Bruining, H.A.; Puppels, G.J. In vivo confocal Raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles. *The Journal of investigative dermatology* **2001**, *116*, 434-442, doi:10.1046/j.1523-1747.2001.01258.x.
40. Choe, C.; Lademann, J.; Darvin, M.E. Analysis of Human and Porcine Skin in vivo/ex vivo for Penetration of Selected Oils by Confocal Raman Microscopy. *Skin pharmacology and physiology* **2015**, *28*, 318-330, doi:10.1159/000439407.
41. Lohan, S.B.; Saeidpour, S.; Solik, A.; Schanzer, S.; Richter, H.; Dong, P.; Darvin, M.E.; Bodmeier, R.; Patzelt, A.; Zoubari, G.; Unbehauen, M.; Haag, R.; Lademann, J.; Teutloff, C.; Bittl, R.; Meinke, M.C. Investigation of the cutaneous penetration behavior of dexamethasone loaded to nano-sized lipid particles by EPR spectroscopy, and confocal Raman and laser scanning microscopy. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V* **2017**, *116*, 102-110, doi:10.1016/j.ejpb.2016.12.018.
42. Darvin, M.E.; Meinke, M.C.; Sterry, W.; Lademann, J. Optical methods for noninvasive determination of carotenoids in human and animal skin. *Journal of biomedical optics* **2013**, *18*, 61230, doi:10.1117/1.Jbo.18.6.061230.
43. Chopra, R.; Vakharia, P.P.; Sacotte, R.; Patel, N.; Immaneni, S.; White, T.; Kantor, R.; Hsu, D.Y.; Silverberg, J.I. Severity strata for Eczema Area and Severity Index (EASI), modified EASI, Scoring Atopic Dermatitis (SCORAD), objective SCORAD, Atopic Dermatitis Severity Index and body surface area in adolescents and adults with atopic dermatitis. *The British journal of dermatology* **2017**, *177*, 1316-1321, doi:10.1111/bjd.15641.
44. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology (Basel, Switzerland)* **1993**, *186*, 23-31, doi:10.1159/000247298.
45. Odhiambo, J.A.; Williams, H.C.; Clayton, T.O.; Robertson, C.F.; Asher, M.I. Global variations in prevalence of eczema symptoms in children from ISAAC Phase Three. *The Journal of allergy and clinical immunology* **2009**, *124*, 1251-1258.e1223, doi:10.1016/j.jaci.2009.10.009.
46. Kim, D.H.; Li, K.; Seo, S.J.; Jo, S.J.; Yim, H.W.; Kim, C.M.; Kim, K.H.; Kim, D.W.; Kim, M.B.; Kim, J.W.; Ro, Y.S.; Park, Y.L.; Park, C.W.; Lee, S.C.; Cho, S.H. Quality of life and disease severity are correlated in patients with atopic dermatitis. *Journal of Korean medical science* **2012**, *27*, 1327-1332, doi:10.3346/jkms.2012.27.11.1327.
47. Eichenfield, L.F.; Ahluwalia, J.; Waldman, A.; Borok, J.; Udkoff, J.; Boguniewicz, M. Current guidelines for the evaluation and management of atopic dermatitis: A comparison of the Joint Task Force Practice Parameter and American Academy of Dermatology guidelines. *The Journal of allergy and clinical immunology* **2017**, *139*, S49-s57, doi:10.1016/j.jaci.2017.01.009.
48. Mohan, G.C.; Lio, P.A. Comparison of Dermatology and Allergy Guidelines for Atopic Dermatitis Management. *JAMA dermatology* **2015**, *151*, 1009-1013, doi:10.1001/jamadermatol.2015.0250.
49. Weidinger, S.; Baurecht, H.; Schmitt, J. A 5-year randomized trial on the safety and efficacy of pimecrolimus in atopic dermatitis: a critical appraisal. *The British journal of dermatology* **2017**, *177*, 999-1003, doi:10.1111/bjd.15827.
50. Ji, H.; Li, X.K. Oxidative Stress in Atopic Dermatitis. *Oxidative medicine and cellular longevity* **2016**, *2016*, 2721469, doi:10.1155/2016/2721469.

51. Chung, J.; Oh, S.Y.; Shin, Y.K. Association of glutathione-S-transferase polymorphisms with atopic dermatitis risk in preschool age children. *Clinical chemistry and laboratory medicine* **2009**, *47*, 1475-1481, doi:10.1515/cclm.2009.336.
52. Niwa, Y.; Sumi, H.; Kawahira, K.; Terashima, T.; Nakamura, T.; Akamatsu, H. Protein oxidative damage in the stratum corneum: Evidence for a link between environmental oxidants and the changing prevalence and nature of atopic dermatitis in Japan. *The British journal of dermatology* **2003**, *149*, 248-254, doi:10.1046/j.1365-2133.2003.05417.x.
53. Ahn, K. The role of air pollutants in atopic dermatitis. *The Journal of allergy and clinical immunology* **2014**, *134*, 993-999; discussion 1000, doi:10.1016/j.jaci.2014.09.023.
54. Hoffmann, J.; Gendrisch, F.; Schempp, C.M.; Wölfle, U. New Herbal Biomedicines for the Topical Treatment of Dermatological Disorders. *Biomedicines* **2020**, *8*, doi:10.3390/biomedicines8020027.
55. Zink, A.; Traidl-Hoffmann, C. Green tea in dermatology--myths and facts. *Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology : JDDG* **2015**, *13*, 768-775, doi:10.1111/ddg.12737.
56. Hwang, Y.; Chang, B.; Kim, T.; Kim, S. Ameliorative effects of green tea extract from tannase digests on house dust mite antigen-induced atopic dermatitis-like lesions in NC/Nga mice. *Archives of dermatological research* **2019**, *311*, 109-120, doi:10.1007/s00403-018-01886-6.
57. Bogdan Allemann, I.; Baumann, L. Antioxidants used in skin care formulations. *Skin therapy letter* **2008**, *13*, 5-9.
58. Zuercher, A.W.; Holvoet, S.; Weiss, M.; Mercenier, A. Polyphenol-enriched apple extract attenuates food allergy in mice. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology* **2010**, *40*, 942-950, doi:10.1111/j.1365-2222.2010.03460.x.
59. Akazome, Y. Characteristics and physiological functions of polyphenols from apples. *BioFactors (Oxford, England)* **2004**, *22*, 311-314, doi:10.1002/biof.5520220161.
60. Meinke, M.C.; Nowbary, C.K.; Schanzer, S.; Vollert, H.; Lademann, J.; Darvin, M.E. Influences of Orally Taken Carotenoid-Rich Curly Kale Extract on Collagen I/Elastin Index of the Skin. *Nutrients* **2017**, *9*, doi:10.3390/nu9070775.
61. Meinke, M.C.; Friedrich, A.; Tscherch, K.; Haag, S.F.; Darvin, M.E.; Vollert, H.; Groth, N.; Lademann, J.; Rohn, S. Influence of dietary carotenoids on radical scavenging capacity of the skin and skin lipids. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V* **2013**, *84*, 365-373, doi:10.1016/j.ejpb.2012.11.012.
62. Sekiguchi, A.; Motegi, S.I.; Fujiwara, C.; Yamazaki, S.; Inoue, Y.; Uchiyama, A.; Akai, R.; Iwawaki, T.; Ishikawa, O. Inhibitory effect of kaempferol on skin fibrosis in systemic sclerosis by the suppression of oxidative stress. *Journal of dermatological science* **2019**, *96*, 8-17, doi:10.1016/j.jdermsci.2019.08.004.
63. Özay, Y.; Güzel, S.; Yumrutaş, Ö.; Pehlivanoğlu, B.; Erdoğdu İ, H.; Yildirim, Z.; Türk, B.A.; Darcan, S. Wound Healing Effect of Kaempferol in Diabetic and Nondiabetic Rats. *The Journal of surgical research* **2019**, *233*, 284-296, doi:10.1016/j.jss.2018.08.009.
64. Al-Madhagy, S.A.; Mostafa, N.M.; Youssef, F.S.; Awad, G.E.A.; Eldahshan, O.A.; Singab, A.N.B. Metabolic profiling of a polyphenolic-rich fraction of Coccinia

grandis leaves using LC-ESI-MS/MS and in vivo validation of its antimicrobial and wound healing activities. *Food & function* **2019**, *10*, 6267-6275, doi:10.1039/c9fo01532a.
65. Podda, M.; Zollner, T.M.; Grundmann-Kollmann, M.; Thiele, J.J.; Packer, L.; Kaufmann, R. Activity of alpha-lipoic acid in the protection against oxidative stress in skin. *Current problems in dermatology* **2001**, *29*, 43-51, doi:10.1159/000060652.
66. Candi, E.; Schmidt, R.; Melino, G. The cornified envelope: a model of cell death in the skin. *Nature reviews. Molecular cell biology* **2005**, *6*, 328-340, doi:10.1038/nrm1619.
67. Seifter, E.; Rettura, G.; Barbul, A.; Levenson, S.M. Arginine: an essential amino acid for injured rats. *Surgery* **1978**, *84*, 224-230.
68. Blecher, K.; Martinez, L.R.; Tuckman-Vernon, C.; Nacharaju, P.; Schairer, D.; Chouake, J.; Friedman, J.M.; Alfieri, A.; Guha, C.; Nosanchuk, J.D.; Friedman, A.J. Nitric oxide-releasing nanoparticles accelerate wound healing in NOD-SCID mice. *Nanomedicine : nanotechnology, biology, and medicine* **2012**, *8*, 1364-1371, doi:10.1016/j.nano.2012.02.014.
69. Schempp, C.M.; Schöpf, E.; Simon, J.C. [Plant-induced toxic and allergic dermatitis (phytodermatitis)]. *Der Hautarzt; Zeitschrift fur Dermatologie, Venerologie, und verwandte Gebiete* **2002**, *53*, 93-97, doi:10.1007/s001050100212.
70. Haag, S.F.; Tscherch, K.; Arndt, S.; Kleemann, A.; Gersonde, I.; Lademann, J.; Rohn, S.; Meinke, M.C. Enhancement of skin radical scavenging activity and stratum corneum lipids after the application of a hyperforin-rich cream. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V* **2014**, *86*, 227-233, doi:10.1016/j.ejpb.2013.06.016.
71. Darvin, M.E.; Fluhr, J.W.; Schanzer, S.; Richter, H.; Patzelt, A.; Meinke, M.C.; Zastrow, L.; Golz, K.; Doucet, O.; Sterry, W.; Lademann, J. Dermal carotenoid level and kinetics after topical and systemic administration of antioxidants: enrichment strategies in a controlled in vivo study. *Journal of dermatological science* **2011**, *64*, 53-58, doi:10.1016/j.jdermsci.2011.06.009.
72. Lademann, J.; Vergou, T.; Darvin, M.E.; Patzelt, A.; Meinke, M.C.; Voit, C.; Papakostas, D.; Zastrow, L.; Sterry, W.; Doucet, O. Influence of Topical, Systemic and Combined Application of Antioxidants on the Barrier Properties of the Human Skin. *Skin pharmacology and physiology* **2016**, *29*, 41-46, doi:10.1159/000441953.
73. Mitamura, Y.; Nunomura, S.; Furue, M.; Izuhara, K. IL-24: A new player in the pathogenesis of pro-inflammatory and allergic skin diseases. *Allergology international : official journal of the Japanese Society of Allergology* **2020**, *69*, 405-411, doi:10.1016/j.alit.2019.12.003.
74. Kamsteeg, M.; Zeeuwen, P.L.; de Jongh, G.J.; Rodijk-Olthuis, D.; Zeeuwen-Franssen, M.E.; van Erp, P.E.; Schalkwijk, J. Increased expression of carbonic anhydrase II (CA II) in lesional skin of atopic dermatitis: regulation by Th2 cytokines. *The Journal of investigative dermatology* **2007**, *127*, 1786-1789, doi:10.1038/sj.jid.5700752.
75. Suri, B.K.; Verma, N.K.; Schmidtchen, A. Toll-like Receptor 3 Agonist, Polyinosinic-polycytidylic Acid, Upregulates Carbonic Anhydrase II in Human Keratinocytes. *Acta dermato-venereologica* **2018**, *98*, 762-765, doi:10.2340/00015555-2963.
76. Blome, C.; Radtke, M.A.; Eissing, L.; Augustin, M. Quality of Life in Patients with Atopic Dermatitis: Disease Burden, Measurement, and Treatment Benefit.

American journal of clinical dermatology **2016**, *17*, 163-169, doi:10.1007/s40257-015-0171-3.
77. Yosipovitch, G.; Fleischer, A. Itch associated with skin disease: advances in pathophysiology and emerging therapies. *American journal of clinical dermatology* **2003**, *4*, 617-622, doi:10.2165/00128071-200304090-00004.
78. Damien, F.; Boncheva, M. The extent of orthorhombic lipid phases in the stratum corneum determines the barrier efficiency of human skin in vivo. *The Journal of investigative dermatology* **2010**, *130*, 611-614, doi:10.1038/jid.2009.272.
79. Eberlein-König, B.; Schäfer, T.; Huss-Marp, J.; Darsow, U.; Möhrenschlager, M.; Herbert, O.; Abeck, D.; Krämer, U.; Behrendt, H.; Ring, J. Skin surface pH, stratum corneum hydration, trans-epidermal water loss and skin roughness related to atopic eczema and skin dryness in a population of primary school children. *Acta dermato-venereologica* **2000**, *80*, 188-191, doi:10.1080/000155500750042943.
80. Blank, I.H.; Shappirio, E.B. The water content of the stratum corneum. III. Effect of previous contact with aqueous solutions of soaps and detergents. *The Journal of investigative dermatology* **1955**, *25*, 391-401.
81. Choe, C.; Schleusener, J.; Lademann, J.; Darvin, M.E. Keratin-water-NMF interaction as a three layer model in the human stratum corneum using in vivo confocal Raman microscopy. *Scientific reports* **2017**, *7*, 15900, doi:10.1038/s41598-017-16202-x.
82. Nakazawa, H.; Ohta, N.; Hatta, I. A possible regulation mechanism of water content in human stratum corneum via intercellular lipid matrix. *Chemistry and physics of lipids* **2012**, *165*, 238-243, doi:10.1016/j.chemphyslip.2012.01.002.
83. van Smeden, J.; Janssens, M.; Gooris, G.S.; Bouwstra, J.A. The important role of stratum corneum lipids for the cutaneous barrier function. *Biochimica et biophysica acta* **2014**, *1841*, 295-313, doi:10.1016/j.bbalip.2013.11.006.
84. Long, S.A.; Wertz, P.W.; Strauss, J.S.; Downing, D.T. Human stratum corneum polar lipids and desquamation. *Archives of dermatological research* **1985**, *277*, 284-287, doi:10.1007/bf00509081.

Statutory Declaration

"I,Yu Zhang, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic: Study of the efficacy of a new topical skin formulation rich in antioxidants in patients with mild to moderate atopic dermatitis/Studie über die Wirksamkeit einer neuen, antioxidantienreichen topischen Hautrezeptur bei Patienten mit leichter bis mittelschwerer Atopic Dermatitis, independently and without the support of third parties, and that I used no other sources and aids than those stated.
All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; http://www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

Date Signature

Declaration of your own contribution to the publications

Yu Zhang contributed the following to the below listed publications:

Publication 1:Yu Zhang , Nina Heinemann , Franziska Rademacher , Maxim E. Darvin , Christian Raab , Cornelia M. Keck , Henning Vollert , Joachim W. Fluhr , Regine Gläser , Jürgen Harder and Martina C. Meinke ,Skin Care Product Rich in Antioxidants and Anti-Inflammatory Natural Compounds Reduces Itching and Inflammation in the Skin of Atopic Dermatitis Patients,Antioxidants,2022

- Identification of clinical assessment indicators and creation of clinical measurement forms
- Perform all measurements (except: sample preparation and in vitro research on cell culture)
- Create all figures and tables (except: table 2, figure 1, figure 2)
- Statistical evaluation (except: data from cell culture)
- Data analysis and results evaluation
- Manuscript writing (except: methods for cell culture)
- Journal revisions

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Signature, date and stamp of first supervising university professor / lecturer

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Signature of doctoral candidate

Excerpt from Journal Summary List

Journal Data Filtered By: **Selected JCR Year: 2021** Selected Editions: SCIE,SSCI Selected Categories: **"BIOCHEMISTRY and MOLECULAR BIOLOGY"** Selected Category Scheme: WoS

Gesamtanzahl: 296 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfaktor
1	NATURE MEDICINE	141,857	87.241	0.23255
2	CELL	362,236	66.850	0.53397
3	Molecular Cancer	32,250	41.444	0.03386
4	Signal Transduction and Targeted Therapy	11,026	38.104	0.01781
5	Annual Review of Biochemistry	25,139	27.258	0.01962
6	Molecular Plant	20,242	21.949	0.02339
7	MOLECULAR CELL	94,258	19.328	0.13937
8	NUCLEIC ACIDS RESEARCH	284,490	19.160	0.33755
9	NATURE STRUCTURAL & MOLECULAR BIOLOGY	33,999	18.361	0.04689
10	TRENDS IN MICROBIOLOGY	19,957	18.230	0.02015
11	CYTOKINE & GROWTH FACTOR REVIEWS	9,002	17.660	0.00625
12	MOLECULAR ASPECTS OF MEDICINE	8,986	16.337	0.00615
13	Nature Chemical Biology	31,125	16.174	0.04456
14	TRENDS IN MOLECULAR MEDICINE	14,585	15.272	0.01381
15	NATURAL PRODUCT REPORTS	14,564	15.111	0.01079
16	PROGRESS IN LIPID RESEARCH	7,982	14.673	0.00444
17	TRENDS IN BIOCHEMICAL SCIENCES	22,957	14.264	0.02170
18	EMBO JOURNAL	80,536	14.012	0.05438
19	MOLECULAR PSYCHIATRY	33,324	13.437	0.04914
20	Molecular Systems Biology	11,036	13.068	0.01483
21	EXPERIMENTAL AND MOLECULAR MEDICINE	12,199	12.153	0.01698

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfaktor
22	PLANT CELL	67,319	12.085	0.02964
23	CELL DEATH AND DIFFERENTIATION	31,035	12.067	0.02639
24	BIOCHIMICA ET BIOPHYSICA ACTA-REVIEWS ON CANCER	8,255	11.414	0.00673
25	Cell Systems	8,047	11.091	0.03332
26	CURRENT BIOLOGY	85,124	10.900	0.10641
27	Redox Biology	20,557	10.787	0.02390
28	International Journal of Biological Sciences	14,100	10.750	0.01488
29	MATRIX BIOLOGY	9,415	10.447	0.00856
30	PLOS BIOLOGY	44,888	9.593	0.05920
31	Cell and Bioscience	4,564	9.584	0.00524
32	Science Signaling	17,426	9.517	0.02046
33	GENOME RESEARCH	51,169	9.438	0.05153
34	CELLULAR AND MOLECULAR LIFE SCIENCES	38,745	9.207	0.03204
35	Journal of Integrative Plant Biology	8,456	9.106	0.00730
36	EMBO REPORTS	21,705	9.071	0.02695
37	Cell Chemical Biology	6,651	9.039	0.01870
38	CURRENT OPINION IN CHEMICAL BIOLOGY	12,464	8.972	0.01277
39	MOLECULAR BIOLOGY AND EVOLUTION	67,311	8.800	0.07228
40	ONCOGENE	81,646	8.756	0.05014
41	CELLULAR & MOLECULAR BIOLOGY LETTERS	2,684	8.702	0.00250
42	CRITICAL REVIEWS IN BIOCHEMISTRY AND MOLECULAR BIOLOGY	5,108	8.697	0.00477
43	Molecular Ecology Resources	15,145	8.678	0.01553

Selected JCR Year: 2021; Selected Categories: "BIOCHEMISTRY and MOLECULAR BIOLOGY"

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfaktor
44	Plant Communications	743	8.625	0.00148
45	FREE RADICAL BIOLOGY AND MEDICINE	55,523	8.101	0.02824
46	INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES	112,372	8.025	0.09445
47	Biomedical Journal	2,388	7.892	0.00301
48	CURRENT OPINION IN STRUCTURAL BIOLOGY	13,407	7.786	0.01689
49	AMERICAN JOURNAL OF RESPIRATORY CELL AND MOLECULAR BIOLOGY	16,259	7.748	0.01386
50	Antioxidants	21,453	7.675	0.01946
51	EXPERT REVIEWS IN MOLECULAR MEDICINE	2,282	7.615	0.00062
52	Reviews of Physiology Biochemistry and Pharmacology	920	7.500	0.00043
53	ANTIOXIDANTS & REDOX SIGNALING	29,117	7.468	0.01390
54	Essays in Biochemistry	4,569	7.258	0.00691
55	Genes & Diseases	2,732	7.243	0.00322
56	Open Biology	5,227	7.124	0.00994
57	PROTEIN SCIENCE	18,673	6.993	0.02822
58	BIOMACROMOLECULES	46,963	6.978	0.02347
59	JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B-BIOLOGY	18,610	6.814	0.01229
60	JOURNAL OF LIPID RESEARCH	29,128	6.676	0.01485
61	BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR BASIS OF DISEASE	22,719	6.633	0.01820
62	MOLECULAR ECOLOGY	45,664	6.622	0.03311
63	AMYLOID-JOURNAL OF PROTEIN FOLDING DISORDERS	2,335	6.571	0.00312

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Skin Care Product Rich in Antioxidants and Anti-Inflammatory Natural Compounds Reduces Itching and Inflammation in the Skin of Atopic Dermatitis Patients

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Abstract: The atopic dermatitis (AD) complex pathogenesis mechanism reveals marked changes of certain signaling factors as well as some morphological alterations in the epidermis. Reduced resilience against environmental factors and oxidative stress often makes the treatment with corticosteroids or tacrolismus ointments indispensable. In view of the correlation between oxidative stress and AD pathological factors, antioxidants can be incorporated into AD management strategies. This study investigates a curly kale, apple and green tea-containing natural extract rich in antioxidants for its effects on signaling inflammatory molecules and skin barrier enhancement in human epidermal keratinocytes- (NHEKs) based cell assays. Furthermore, the skin penetration on porcine ears was measured ex vivo using Raman micro spectroscopy. Finally, in a double-blind half-side, placebo-controlled clinical study, the effects of a formulation containing this extract were analyzed for the influence of lesion severity, epidermal barrier function, and pruritus in mild to moderately AD patients. Summarizing our results: The extract reduces expression of inflammatory cytokines in keratinocytes and increases barrier-related molecules. The verum formulation with a very high antioxidant capacity used in AD patients with mild to moderate lesions reduces itching, local SCORAD, and improves barrier function and the hydration of skin lesions.

Keywords: atopic dermatitis; green tea; apple extract; Raman spectroscopy; stratum corneum

1. Introduction

Atopic dermatitis (AD) is a common inflammatory skin disease characterized by epidermal dysfunction. As a common disease with high prevalence on a global scale, it has a significant impact on patients' quality of life [1]. The pathogenesis of AD is complex, resulting from an interaction of genetic, immunologic and environmental factors [2].

The human epidermis includes the following main layers: basal layer on the basement membrane, spinous, granular and lucidum layers, and the outermost cornified layer—stratum corneum. The stratum lucidum is usually found in areas of thick skin, i.e., the

hands, palms, and the soles of the feet. Keratinocytes are the most numerous cells in the epidermis, which are continuously differentiated from the basal layer towards the stratum corneum. Different layers together constitute the multi-tiered barrier of human skin to prevent the loss of water and the infiltration of environmental allergens and microbial pathogens.

Upon terminal differentiation, keratinocytes synthesize several of the barrier-related molecules, including keratin-1 and -10, loricrin, filaggrin, and involucrin [3], hydrolytic enzymes, phospholipids, ceramides, glycosyl ceramides and sterols [4].

These molecules play an important role in maintaining the integrity of the skin's barrier function.

In AD patients, it can be observed that the expression of terminal differentiation molecules, such as filaggrin, involucrin and loricrin, is reduced. This downregulation is one of the influencing factors that cause skin barrier dysfunction [5]. Loss-of-function mutations in filaggrin have been identified as important genetic determinants for AD development [6,7].

In addition to defects in terminal epithelial differentiation such as lack of filaggrin, immune dysregulation, which is mainly a type 2 response, is also one of the factors leading to skin barrier dysfunction [2]. Skin barrier dysfunction, scratching, bacterial colonization and other factors inducing a type 2 immune response leading to the overexpression of IL-13 and IL-4 produced by T-helper 2(Th2) lymphocytes. IL-13 and IL-4 inhibit the expression of filaggrin by up-regulating the expression of IL-24, increasing the production of chemokines, such as CCL17, CCL22 and CCL26, magnifying IL-31-induced pruritus and down-regulating the expression of antimicrobial peptides [2,8].

Oxidative stress, which refers to the generation of reactive oxygen species (ROS) that exceed the scavenging capacity of the antioxidant system (AOS), plays a crucial role in chronic inflammatory skin diseases such as AD [9]. In AD, oxidative stress caused by Th2 cytokines leads to sustained STAT6 signaling, thus further aggravating the disease symptoms [10].

The basic management strategy of AD is to improve symptoms and establish long-term disease control [11], including reducing acute inflammatory symptoms, restoring skin barrier homeostasis, and avoiding related factors that trigger or aggravate the disease [12].

For most patients with mild AD, topical therapy can control skin inflammation, reduce symptoms and prevent flares [13]. Topical corticosteroids and topical calcineurin inhibitors are first-line treatments for anti-inflammatory and flare reduction; however, they are generally not recommended for long-term daily use due to potential local side effects [14–17]. Thus, despite the current availability of topical treatments for AD, a new topical formulation is required that could enable a balance between efficacy and safety.

In this study, firstly, the anti-inflammatory and barrier enhancement effects of a new extract were studied in cell culture. The radical scavenging activity was investigated for the extract incorporated into a DAC (Deutscher Arzneimittel-Codex)-base cream directly. Subsequently, the skin penetration efficiency of the cream was studied in ex vivo pig ear skin to ensure the uptake of the formulation.

Finally, in a double-blind half-side, placebo-controlled human in vivo study, we investigated the effects of a formulation containing this extract regarding reducing the lesion severity, improvement of epidermal barrier function, and pruritus relief in mild to moderately AD patients.

2. Materials and Methods

2.1. Materials

The extract was obtained from Bioactive Food GmbH (Bad Segeberg, Germany) and contained equal amounts of curly kale extract (Anklam Extract GmbH, Anklam, Germany), green tea extract (Eurochem Feinchemie GmbH, Gröbenzell, Germany), apple extract (Herbstreith & Fox KG, Neuenbürg/Württ, Germany) and L-arginin (Roth, Karlsruhe,

Germany). The flavonoid extracts were prepared using a polymeric adsorbent (Amberlite XAD) according to the study by Zessner et al. [18].

Within the project several commercially available plant extracts (food grade) were preselected based on published data indicating potential beneficial effects on the skin barrier. Kaempferol, a natural flavonol present in various edible plants (including kale), has been reported to possess various anti-inflammatory properties [19]. Kaempferol reduced gene expression of major proinflammatory cytokines, including interleukin (IL)-6, IL-17A and tumor necrosis factor (TNF)-α, in the psoriatic skin lesion [20]. However, it is unknown whether kaempferol as well as kaempferol flavonoids would have an effect in AD. Various biological activities of catechins, the main constituent of green tea, including antioxidant and anti-inflammatory properties, have been reported [21,22]. Furthermore, apple flavonoids like phlorizin were effective in protecting the skin against UVB-induced skin damage by decreasing ROS overproduction, Cox-2 expression and the subsequent excessive inflammation reactions [23]. L-arginine was added to improve the skin barrier factors and moisten the skin in vivo.

For the ex vivo and in vivo experiments, creams that contained 2% (*w*/*w*) extract in base cream DAC (verum) were produced (Löwen-Apotheke, Bad Segeberg, Germany), and cream base without extract was used as control (placebo). Table 1 provides an overview of the formulations used in this study.

Table 1. Composition of verum and placebo creams.

Ingredients	Extract	DAC Basis Creme
	• 25.0 g curly kale extract (30% kaempferol flavonoids) • 25.0 g green tea extract (60% epigallocatechin gallate) • 25.0 g apple extract (15% phlorizin, 5% quercetin flavonoids) • 25.0 g L-arginine	• 4.0 g glycerol monostearate 60 • 6.0 g cetylalcohol • 7.5 g medium-chain trigylcerides (mygliol®812, neutral oil) • 25.5 g white vaseline (petroleum jelly) • 7.0 g macrogol-20-glycerol monostearate • 10.0 g propylene glycol • 40.0 g purified water
Verum (*w*/*w*)	2%	98%
Placebo (*w*/*w*)	0%	100%

2.2. In Vitro Experiments on Cell Culture

Pooled primary normal human epidermal keratinocytes (NHEKs), isolated from the epidermis of juvenile foreskin of three male caucasian donors, were used for the in vitro experiments (Lot Number: 456Z001.1, PromoCell, Heidelberg). NHEKs at passage 4 were seeded in a 24-well plate in Keratinocyte Growth Medium 2 (KGM2) (PromoCell, Heidelberg, Germany) supplemented with KGM2 Supplement Mix including $CaCl_2$ (PromoCell). After reaching 90–100% confluency, cells were incubated in a medium containing 1.3 mM $CaCl_2$ for 48 h to induce differentiation. Differentiated NHEKs were stimulated with a cytokine mix (IL-22 (10 ng/mL), tumor necrosis factor (TNF) alpha (10 ng/mL), IL-13 (10 ng/mL) and IL-4 (10 ng/mL)) to mimic an AD-like inflammation [24]. Simultaneously, these cells were incubated with a 1:800 dilution of the plant extract mixture (10 mg/mL kale extract, 10 mg/mL green tea extract, 10 mg/mL apple extract, 10 mg/mL L-arginine in 50% ethanol) or vehicle control (50% ethanol) for 20 h at 37 °C in 5% CO_2 atmosphere.

Real-Time PCR

After stimulation, the gene expression levels of AD-relevant genes were determined by real-time PCR. Therefore, total RNA was isolated using the Crystal RNAmagic kit (Biolabproducts, Bebensee, Germany). Isolated RNA was transcribed to cDNA using a PrimeScript reverse transcriptase kit (Takara Bio, Saint-Germain-en-Laye, France). Subsequent analysis by real-time PCR was performed with the QuantStudio3 System (Thermo Fisher

Scientific, Schwerte, Germany) using the temperature profile as described previously [25]. Gene expression levels of carbonic anhydrase II (CA2, gene acc. no.: NM_000067.2), CC-chemokine ligand 26 (CCL26, gene acc. no.: NM_006072.4), collagen type I alpha 1 chain (COL1A1, gene acc. no.: NM_000088.3), filaggrin (FLG, gene acc. no.: NM_002016.1), Interleukin-24 (IL-24, gene acc. no.: NM_006850.3), involucrin (IVL, gene acc. no.: NM_005547.2), loricrin (LOR, gene acc. no.: NM_000427.2) and homo sapiens ribosomal protein L38 (RPL38, acc. no.: NM_000999.3) were measured. The following intron-spanning primers were used (Table 2):

Table 2. Primer sequences used in the real-time PCR to determine the gene expression level of AD-relevant genes.

Gene	Forward Primer	Reverse Primer
Carbonic Anhydrase II, CA2	5′-AACAATGGTCATGCTTTCAACG-3′	5′-TGTCCATCAAGTGAACCCCAG-3′
CC-chemokine ligand 26, CCL26,	5′-AATTGAGGCTGAGCCAAAGA-3′	5′-ATCAGGCCCTTCTCAGGTTT-3′
Collagen, type 1, alpha 1, COL1A1	5′-CTGGAAGAGTGGAGAGTACTG	5′-GTCTCCATGTTGCAGAAGAC-3′
Filaggrin, FLG	5′-GGCAAATCCTGAAGAATCCAGATG-3′	5′-GGTAAATTCTCTTTTCTGGTAGACTC-3′
Interleukin-24, IL-24	5′-GTTCCCCAGAAACTGTGGGA-3′	5′-CGAGACGTTCTGCAGAACC-3′
Involucrin, IVL	5′-GGAGGAGGAACAGTCTTGAGG-3′	5′-CTGCCTCAGCCTTACTGTGA-3′
Loricirin, LOR	5′-CTCTCCTCACTCACCCTTCCT-3′	5′-AGGTCTTCACGCAGTCCAC-3′
Homo sapiens ribosomal protein L38, RPL38	5′-TCAAGGACTTCCTGCTCACA-3′	5′-AAAGGTATCTGCTGCATCGAA-3′

The gene expression levels were normalized to the constitutively expressed ribosomal protein L38 (RPL38) gene.

2.3. Radical Protection Factor (RPF)

The RPF technology can determine the antioxidant capacity of the sample by measuring the free radical scavenging activity [26]. Thus, we used electron paramagnetic resonance (EPR) spectroscopy (X-band EPR spectrometer (9.4 GHz) MiniScope MS5000, Magnettech, Freiberg Instruments, Freiberg, Germany) to determine the RPF of the formulation by measuring the scavenged marker radicals. In this study, the following EPR settings were used: modulation amplitude 2 G, microwave (MW) attenuation 15 dB (MW power 3.16 mW), sweep time 20 s, sweep 95 G, B0-field 3350 G, step 1024 and number(pass) 1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, Steinheim, Germany) was used as a test radical. The signal intensity in the sample is determined by the concentration of DPPH. The lower the DPPH concentration, the weaker the signal and the more active the radical-scavenging activity of the cream. For the measurements, 50 mg of each formulation was diluted in 10 mL of ethanol, and then 400 µL of this solution was mixed with 400 µL DPPH (1 mM). The samples were kept under constant agitation in the dark at room temperature until the end of the measurements. The measurements started directly after mixing with DPPH, and measurements were carried out every hour until the DPPH signal was stable (approx. 19 h). After measuring the EPR signal strength, we used the following formula to calculate RPF:

$$RPF = \frac{RC\ \ RF}{PI}$$

Whereby RC is the concentration of the test radical, which was measured in radicals per milliliter, RF is the reduction factor, which represents the difference between the untreated test radical intensity and the decreased signal intensity after treatment with the antioxidant normalized to the signal of the untreated test radical, and PI is the product input, which

represents the amount of the substance/product measured in milligrams per milliliter [27]. A positive number N that presents the measuring unit 10^{14} radicals/mg expresses the RPF.

2.4. Ex Vivo Investigations on Porcine Ear Skin

Nine fresh porcine ears obtained from the local slaughterhouse were gently washed with running room temperature water and dried with soft paper towels. The hair was gently cut without damaging the stratum corneum. A marker was used to label 1 cm × 2 cm, subsequently, two creams (verum and placebo creams) were applied at two different doses (1 mg/cm^2 and 2 mg/cm^2) evenly on the marked areas. The treated porcine ears were left at 32 °C and 90% humidity for 2 h and 4 h, respectively, for passive penetration. Subsequently, confocal Raman micro spectroscopy (CRM) was used to observe the 10 processed skin samples. At least eight points on each skin area without any hair or furrows were selected for CRM measurement and subsequent analysis.

2.5. Confocal Raman Microspectroscopy

An in vivo confocal Raman microscope (Model 3510 SCA, RiverD International B.V., Rotterdam, The Netherlands) with an excitation wavelength of 785 nm was applied to the untreated and cream-treated skin. The fingerprint region (400–2000 cm^{-1}) was used to analyze the samples, and the Raman spectra were recorded from the skin surface down to a depth of 40 µm at increments of 2 µm. The acquisition time for one spectrum was 5 s, and the maximal laser power on the skin was 20 mW. The Raman spectra were analyzed using the non-restricted multiple least square fit method available in the 'Skin Tools 2.0' software developed by RiverD International B.V. [28], and the penetration profiles of the semiquantitative concentration of creams were determined similar to that presented in the literature [29]. The penetration depth value was determined as an intersection of cream-treated and untreated profiles (both calculated using 'SkinTools' for certain cream), similar to that used in [30]. The utilized CRM system was described in detail elsewhere [31].

2.6. Volunteers and Study Design

Ten volunteers with mild to moderately severe AD according to the SCORAD scale (<50) [32] aged 18–60 years (seven female and three male; median age 33 years) were enrolled in the study. Positive votes for the experiments have been obtained from the ethics committee of the Charité – Universitätsmedizin Berlin (EA1/207/20), which were conducted according to the Declaration of Helsinki as revised in 2013, and informed consent was obtained from all patients.

Key exclusion criteria included: (i) patients with severely infected lesions in need of oral or intravenous antibiotics and auxiliary therapy; (ii) patients with dermatological diagnoses other than AD; (iii) people who cannot take responsibility for themselves; (iv) pregnancy or lactation.

The study was a four-week, randomized, double-blind, controlled half-side comparison study. The symmetrical parts (e.g., bilateral forearms) of each volunteer were randomized for treatment with verum or placebo cream treatment in the morning and evening for four weeks. Both creams were packaged in the same containers and were coded (A or B) by the pharmacist.

Only one of the two creams contained AO extract. No other moisturizers or topical treatments were used during the study to control variables. Evaluation of the outcome parameters was performed at baseline (D0) and after four weeks (D28).

2.7. Clinical Assessment

The clinical severity scoring was performed by the SCORAD intensity parameters in the test area (local SCORAD, ranges from 0 to 18), includes the assessments of erythema, edema/papulation, oozing/crusts, excoriation, lichenification, dryness on a 0–3 scale (none-0, mild-1, moderate-2 or severe-3) [33].

The itch/sleeplessness intensity was assessed by the volunteers using a visual analogue scale from 0 (no perceptible itch/sleeplessness) to 10 (worst imaginable itch/ sleeplessness) [33].

Transepidermal water loss (TEWL), stratum corneum hydration (capacitance) and erythema were measured using the Tewameter TM 300, the Corneometer CM825 and the Mexameter MX 18 (Courage & Khazaka Electronic GmbH, Cologne, Germany), respectively. None of the patients used any topical skin care product on the test areas at least 24 h before the first test and 12 h before the second. Measurements were taken on the test area of the patients' lesions after 15 min of inactivity and under controlled environmental conditions (room temperature 20–25 °C; relative humidity 40–60%) [34,35].

The assessment result of erythema and capacitance was based on the average value of three measurements on the test area by the same person. The result of TEWL assessment was the average value of 20 consecutive measurements.

2.8. Data Analysis

GraphPad Prism (Version 8) was used for statistical analysis of the in vitro data. Normality was tested by D'Agostino & Pearson omnibus normality test. For data deviating from normality, the non-parametric Kruskal-Wallis test with subsequent Dunn's Multiple Comparison test was used to determine significant differences.

If the data passed normality but did not have equal variances, a Welch's-ANOVA with subsequent Dunett´s T3 Multiple comparison test was used to determine significant differences. *p*-values were marked by asterisks: * $p < 0.05$, ** $p < 0.01$.

For the in vivo investigations intra-group comparisons were obtained by the Wilcoxon signed ranks test, and inter-group comparisons were performed using the Mann–Whitney U-test. Calculations were done using SPSS 25. Differences with *p*-values of less than 0.05 were considered statistically significant.

3. Results

3.1. Radical Scavenging Activity of the Formulations

The RPF for verum, determined by EPR spectroscopy, was $(690 \pm 30) \times 10^{14}$ radicals/mg, while the RPF for placebo was almost 0. This proves that verum has very high radical scavenging activity, indicating high antioxidant properties.

3.2. Beneficial Effects of the Extract in an In Vitro AD Cell Culture Model

The effects of the plant extract were determined using either a normal (I) or an AD (II) cell culture in vitro model.

(I) For simulating healthy skin conditions, untreated differentiated normal human keratinocytes (NHEKs) were used to evaluate the effects of the extract in vitro. Stimulation with the extract led to significant two to three-fold higher gene expressions of the epidermal differentiation markers filaggrin and loricrin and the extracellular matrix molecule collagen type I alpha 1 chain (COL1A1) as compared to unstimulated keratinocytes (Figure 1 a,b,d). No significant upregulation of gene expression was observed for involucrin (Figure 1c). Analysis of the inflammation marker revealed a significant downregulation of IL-24 gene expression (Figure 2a) by the extract. No effects of the extract on the already low gene expression of CCL26 and carbonic anhydrase 2 were seen (Figure 2b,c).

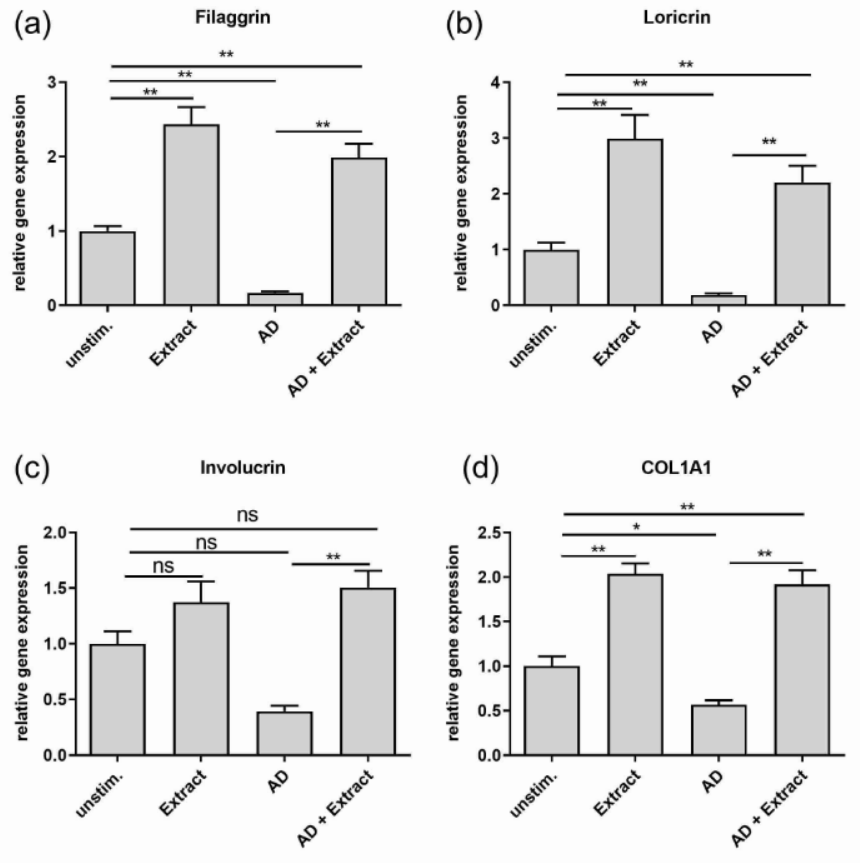

Figure 1. Effect of the plant extract mixture on the gene expression of skin barrier and extracellular matrix molecules in an AD-like in vitro model. NHEKs not treated with specific cytokines were used to reflect healthy skin conditions. These cells were left unstimulated (unstim.) or stimulated with the plant extract (Extract) for 20 h. NHEKs were also treated for 20 h with an AD cytokine mix (IL-22, TNF-alpha, IL-13 and IL-4 (each 10 ng/mL)) either in the absence (AD) or in the presence of the extract (AD + Extract). Gene expression levels of (**a**) filaggrin (**b**) loricrin (**c**) involucrin (**d**) COL1A1 were determined by real-time PCR and normalized to the gene expression of the housekeeping gene RP38. Statistical significances were tested by c) Kruskal-Wallis test with subsequent Dunn's multiple comparison and (**a**,**b**,**d**) Welch's ANOVA with subsequent Dunett´s T3 multiple comparison test. *p*-values were marked by asterisks: * $p < 0.05$, ** $p < 0.01$ (n = 9–18 stimulations).

(II) To evaluate the effects of the extract on AD in vitro, an AD-like model was generated by incubating NHEKs with an AD-typical cytokine mix consisting of IL-22, TNF-alpha, IL-13 and IL-4 [24]. It could be shown that the cytokine mix was able to mimic skin barrier dysregulation as well as an AD-typical inflammation in vitro. First, the skin barrier dysregulation was represented by the significant downregulation of the gene expression of the epidermal differentiation markers filaggrin and loricrin and, by trend, also involucrin ($p = 0.069$) as compared to untreated keratinocytes, which reflect the healthy skin status (Figure 1a–c). In addition, the gene expression of the extracellular matrix protein COL1A1 was significantly downregulated (Figure 1d).

The AD-typical inflammation induced by the AD cytokine mix was seen by the significant and strong upregulation of the gene expression of IL-24, CCL26 and CA2 in comparison to the gene expression in unstimulated cells (Figure 2).

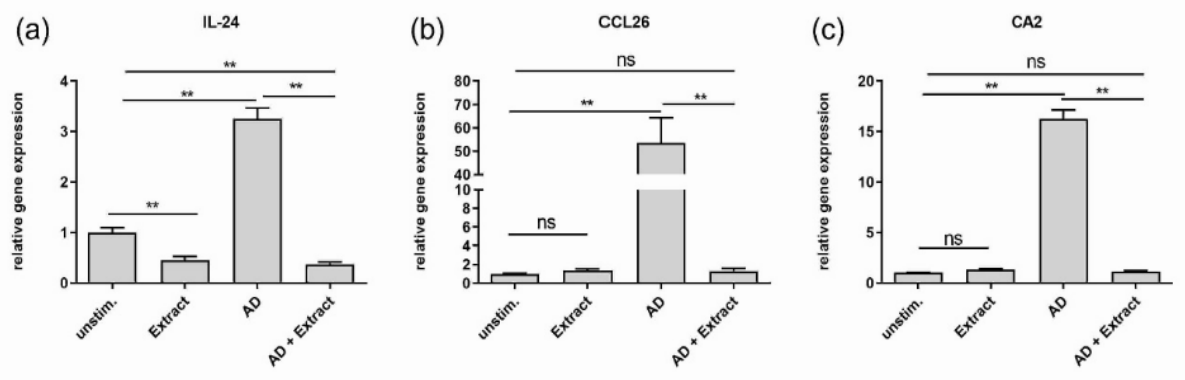

Figure 2. Effect of the plant extract mixture on the gene expression of inflammation marker in an AD-like in vitro model. NHEKs not treated with specific cytokines were used to reflect healthy skin conditions. These cells were left unstimulated (unstim.) or stimulated with the plant extract (Extract) for 20 h. NHEKs were also treated for 20 h with an AD cytokine mix (IL-22, TNF-alpha, IL-13 and IL-4 (each 10 ng/mL)) either in the absence (AD) or in the presence of the extract (AD + Extract). Gene expression levels of (**a**) IL-24 (**b**) CA2 (**c**) CCL26 were determined by real-time PCR and normalized to the gene expression of the housekeeping gene RP38. Statistical significances were tested by (**b**,**c**) Kruskal-Wallis test with subsequent Dunn's multiple comparison and (**a**) Welch's ANOVA with subsequent Dunett´s T3 multiple comparison test. *p*-values were marked by asterisks: ** $p < 0.01$ (n = 9–18 stimulations).

Adding the extract to the AD-like in vitro model had a strong impact on the gene expression profile of skin barrier molecules as well as inflammation markers. On the one hand, the extract restored the downregulated expression of all skin barrier molecules (Figure 1) and reached at least the height of the expression levels of unstimulated cells, or in the case of filaggrin, loricrin and COL1A1, even a significant two-fold higher level (Figure 1a,b,d). On the other hand, the extract downregulated the gene expression of the inflammation marker CCL26 and CA2 significantly to the level of unstimulated cells (Figure 2b,c) or in case of IL-24, even significantly lower, as shown in Figure 2a.

3.3. Penetration Studies

The extract provides high antioxidant and anti-inflammation properties and enhances the skin barrier function. The question arose whether the formulations or parts of it reach the living cells of the epidermis. Representative averaged Raman spectra of verum and placebo creams (Supplementary Figure S1) as well as cream-treated and untreated skin (Supplementary Figure S2) show a large difference in fluorescence intensity characteristic of the verum extract, allowing determination of the penetration depth using the CRM system.

The penetration profiles of the two creams into the skin are shown in Figure 3. For both creams, the concentration of penetrated compounds decreases exponentially from the surface into the skin. There is not enough evidence to show that there is a difference between the verum and the placebo cream in the penetration depth of cream compounds. The typical stratum corneum thickness is approx. 18 µm [29], so compounds from both verum cream and the placebo cream can permeate the entire stratum corneum. The penetration depth of these compounds (both creams) is not significantly related to the penetration time and dosage.

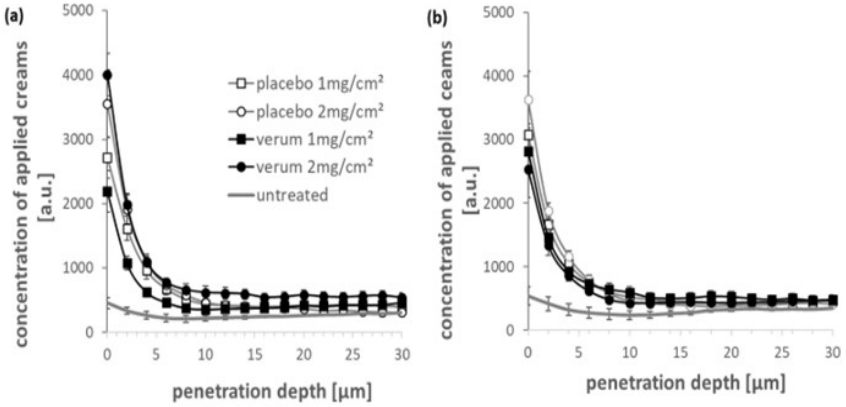

Figure 3. The penetration depth profiles of verum and placebo creams into porcine ear skin obtained after (**a**) 2 and (**b**) 4 h penetration time using CRM compared to untreated skin.

3.4. Clinical Study

The absolute values compared with the baseline of all measured items are shown in Table 3.

Table 3. Clinical and non-invasive bioengineering assessment at baseline (D0) and after four weeks (D28), Mean ± SEM (n = 10).

Skin Parameter	Baseline	Day 28
Local SCORAD		
Verum	6.3 ± 1.0	2.3 ± 0.7
Placebo	5.3 ± 1.0	4.1 ± 0.8
Itch		
Verum	3.8 ± 0.5	1.6 ± 0.4
Placebo	3.9 ± 0.7	3.4 ± 0.8
Sleeplessness		
Verum	2.9 ± 0.9	2.2 ± 0.8
Placebo	2.9 ± 0.9	2.2 ± 0.8
TEWL		
Verum	27 ± 7	16 ± 4
Placebo	27 ± 8	25 ± 7
Skin capacitance		
Verum	20 ± 5	28 ± 5
Placebo	23 ± 5	25 ± 5
Skin erythema		
Verum	340 ± 30	340 ± 40
Placebo	340 ± 40	310 ± 20

The clinical outcomes of local SCORAD, itching and TEWL as a measure of the barrier function are shown in Figure 4.

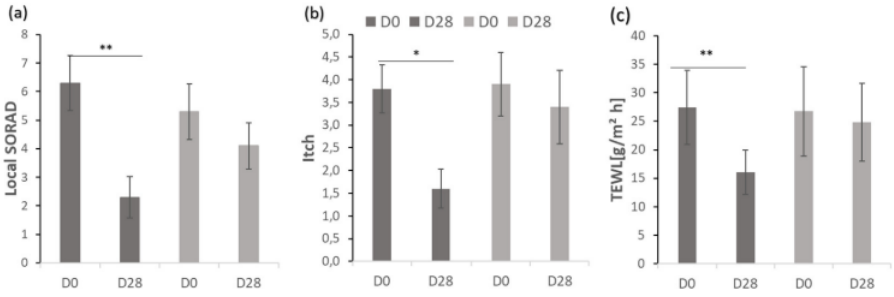

Figure 4. Comparison of (**a**) local SCORAD, (**b**) itch and (**c**) TEWL after four weeks (D28) compared to baseline (D0). Mean ± SEM (n = 10); level of significance * $p < 0.05$, ** $p < 0.01$.

The relative changes to the initial values are shown in Figure 5. In addition to the before presented parameters, sleeplessness, skin hydration and skin erythema were assessed.

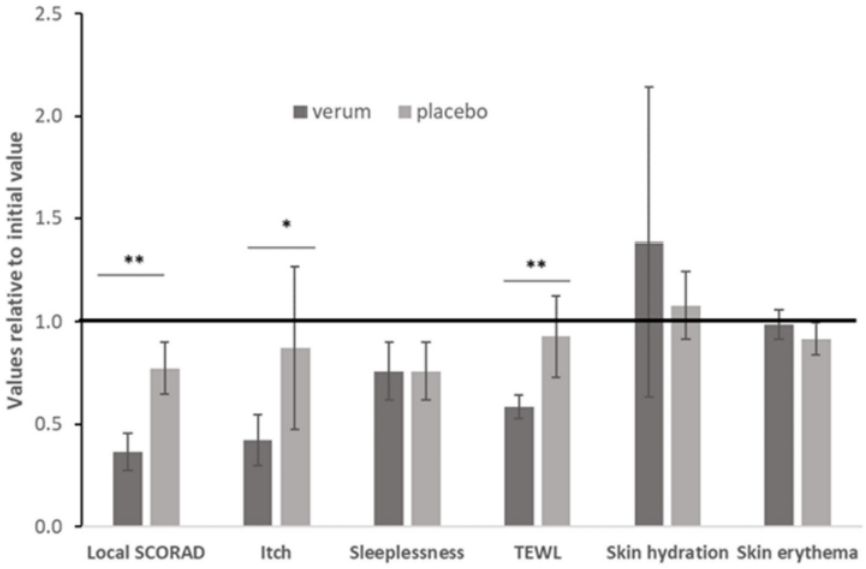

Figure 5. Comparison of the outcomes, assessed as relative value from baseline; level of significance * $p < 0.05$, ** $p < 0.01$.

At baseline (0 week), there were no significant differences in the local severity score, itching intensity and TEWL between the symmetrical test areas. After applying verum and placebo cream, the topical SCORAD was reduced by 63.5 and 22.6%, respectively. For the side where verum cream was applied, the difference in local SCORAD before and after treatment was statistically significant (p = 0.005, Figure 4). The evaluation of the itching intensity showed that after treatment with verum cream, the visual analogue scale values were significantly reduced (p = 0.011, Figure 4). In contrast, the difference before and after the application of the placebo cream was not statistically significant for any of the parameters studied. At 28 days, the TEWL values of the two groups were lower than the baseline. Compared with placebo cream, the difference between before (27.44 ± 6.54, Table 3) and after (16.03 ± 3.92, Table 3) verum cream treatment was more pronounced and statistically different (p = 0.005, Figure 4). Taken together, the application of verum cream can significantly reduce the clinical severity score, the intensity of itching and TEWL, but there is no significant difference before and after the application of placebo.

At baseline (0 week), there were no significant differences in the erythema, sleeplessness intensity, skin hydration (capacitance) between the symmetrically located test areas. Since the evaluation of insomnia is a systemic assessment of the whole body, it is impossible to compare the effects of the two creams, and the application of two creams does not improve the patient's sleep quality (Figure 5). Neither cream can improve or reduce skin erythema (Figure 5). Although there was no statistical difference in skin hydration between the two groups before and after treatment, it can be seen from the average and relative values that the application of verum can increase the hydration of the skin while the placebo cannot (Figure 5 and Table 3). In summary, neither verum nor placebo can reduce erythema, improve sleep or increase skin hydration (capacitance) significantly.

4. Discussion

As mentioned above, the basic treatment strategy for AD patients is to establish a patient-centered long-term management plan to apply moisturizers to restore the skin barrier while increasing or decreasing the use of anti-inflammatory treatments according to the severity of the disease [36,37].

For patients with mild AD, topical therapy is normally usually used to control inflammation, while for patients with moderate to severe AD, it is necessary to consider whether to use systemic therapy according to the international guideline and the individual situation [13].

Topical corticosteroids and calcineurin inhibitors are the first-line for topical anti-inflammatory treatment, although appropriate intermittent use can avoid the risks such as telangiectasia and skin atrophy [38]. For a specific group of people, such as children, AD patients with facial dermatitis, and patients with frequent relapse, there is no doubt that a non-steroidal topical skin care product which has a certain anti-inflammatory effect and repairs the skin barrier is needed.

In recent years, with the exploration of the pathogenesis of atopic dermatitis, the role of oxidative stress in the occurrence and development of AD has gradually emerged. The hallmark pathological feature of AD is chronic dermatitis of no distinctive type. The activation of inflammatory cells increases the production of free radicals, and excess free radicals can also up-regulate the expression of pro-inflammatory cytokines [39]. At the same time, the change in the antioxidant capacity of AD patients cannot be ignored. A study conducted in preschool-age children to explore the relationship between glutathione-S-transferase polymorphisms and atopic dermatitis risk indicated that the reduction of antioxidant capacity in AD patients might play a role in the pathogenesis [40]. Disorders of the patient's antioxidant system can affect the progression of the disease from different aspects, including causing itching, enhancing Th2 polarization, and inducing oxidized protein damage in the stratum corneum [41,42].

Given the correlation between oxidative stress and AD pathological factors, antioxidants can be included in the strategy of AD management. In this study, EPR was used to determine the RPF of the extract, and it was concluded that the verum extract has a very high antioxidant capacity of $(690 \pm 30) \times 10^{14}$ radicals/mg. Most RPF measured for creams so far were far below this value. Nevertheless, already with lower RPF values, strong radical scavenging activities could be found [26,43]. Furthermore, it could be shown that a topically applied ointment containing antioxidants with a high radical protection factor has a number of positive effects on healthy human skin [44,45] and is able to prevent the formation of palmar-plantar erythrodysesthesia which occurs during the chemotherapy of cancer patients [46]. Thus, it is likely that the extract has a certain function of scavenging free radicals in skin lesions which may contribute to the observed effects of the extract. Clearly, more experiments are needed to assess the potential effects of the extract on changes of radicals in skin lesions.

As a barrier organ, the skin is characterized by the ability to selectively allow certain chemicals to pass through the barrier. Most chemicals penetrate the skin through passive diffusion, and few are actively transported. The penetration efficiency of medicinal products

is one of the important factors to improve the efficacy of topical preparations. In this study, using the high molecular specificity of the Raman spectrum, according to the chemical composition of the extract, the CRM measurement method was used to evaluate the penetration depth of the extract into porcine ear skin ex vivo. According to our data (Figure 3), the maximum concentration can be detected near the skin surface, and then the concentration gradually decreases with the depth of detection until it stabilizes. Most of the ingredients remain on the skin surface and in the stratum corneum. Nevertheless, some compounds of both creams could permeate the stratum corneum and might reach the metabolically active layers of the viable epidermis. To prove this experimentally, further investigations are necessary. There is no significant difference between the penetration depth of the penetrated compounds from verum and placebo: basically, it is about 20 µm, which is consistent with the lipid order curve of the stratum corneum [47]. There are no significant differences between the penetration depths for 2 and 4 h of penetration time but after 4 h parts of the cream components could be distributed into the surrounding skin compartments and cannot be distinguished anymore from the skin compounds itself. The penetration efficiency of the verum cream components still has room for improvement. Nevertheless, the penetrated amount of the cream components into the stratum corneum could provide a reservoir function for prolonged diffusion of the actives into the viable epidermis. Tracking the penetration of certain active ingredients is possible using Raman spectra analysis [48–50]. However, due to the low Raman sensitivity of verum extract components compared to the base cream components, we did not focus on individual compounds in this study. We assume that the penetration depth of the active ingredients of the verum extract corresponds to the penetration depth of the detected verum cream components in the skin. This is very likely, since at a skin depth of 20 µm the fluorescence of the extract is still clearly visible (Figure S2). Nevertheless, this should be verified in further investigations.

In in vitro studies, we confirmed that the extract has anti-inflammatory effects. After adding the extract, the expression of IL-24 in the AD model was significantly reduced, even slightly lower than that of the normal skin model. As a member of the IL-20 cytokine family, IL-24 plays a key role in the pathogenesis of AD [51]. IL-24 down-regulates the expression of filaggrin in keratinocytes, contributing to barrier dysfunction downstream of the IL-13/periostin pathway [51].

At the same time, the induction of CCL26, as a chemotactic factor generated through IL-4 and IL-13 stimulation, was significantly reduced by the extract confirming its anti-inflammatory effect, especially for Th2 immune responses. Similarly, the induction of carbonic anhydrase II (CA2) in the AD in vitro model was also completely blocked by the extract. CA2 is an enzyme commonly expressed in a variety of tissues (such as skin, kidneys, red blood cells), and it plays an important role in maintaining cellular pH. Its expression is significantly upregulated in lesioned AD skin, which is in line with the observation that Th2 cytokines induce its expression in keratinocytes. The enhanced expression of CA2 in AD may contribute to abnormally enhanced pH levels seen in AD skin [52,53]. Compared with the side effects of long-term use of topical corticosteroids on the formation of collagen and vessels, the extract can increase the expression of collagen (COL1A1). In addition, it enhances the expression of skin barrier-related proteins (filaggrin, involucrin and loricrin) supporting the restoration of the skin barrier dysfunction.

The above experimental results were further confirmed by an initial four-week clinical study. The clinical study consisted of a double-blind half-sided comparison in 10 patients. The advantage of the half-sided design is that it basically excludes the individual influences of patients' psychological and physiological differences on the test results. From the patient's local SCORAD, it can be seen that compared with placebo cream, the cream with extract significantly improves the symptoms and signs of AD patients. This is attributed to the multiple mechanism of action of the extract: anti-inflammatory/antioxidative effects and skin barrier dysfunction restoration. The improvement of verum on itching degree is also promising. Four weeks after application of the verum, the visual analogue score

(VAS) decreased by more than 50%, while the score of the placebo side was basically the same before and after treatment. In patients with AD, pruritus is the most common yet most difficult symptom to control. The itch-scratch cycle leads to bacterial colonization and continuous inflammation, which further aggravates the skin barrier dysfunction. Additionally, the itching has a non-negligible impact on the quality of life and mental health of patients [54]. The effect of the extract on itching is probably mediated by the restoration of the skin barrier function, which reduces the expression of various pro-inflammatory mediators, including neuropeptides [55]. TEWL is a non-invasive measurement method, which can be used to indirectly evaluate the parameters of the skin barrier function [56]. The use of the extract-containing cream significantly reduced the TEWL of AD patients. Skin dysfunction causes the inability to prevent irritants and itching substances (such as allergens) from entering the skin, thus becoming one of the pathogenesis of itch. The hydration of the stratum corneum is related to the dryness of the skin. The drier the skin, the less skin hydration [57]. The water content in the stratum corneum depends mainly on two aspects: (1) bonding of water molecules by the natural moisturizing factor secondary/tertiary structure of keratin inside the corneocytes [58,59] and bonding of water molecules by hydrophilic parts inside the lipid lamellas [60]; and (2) the barrier formed by the orthorhombic lateral packing order of intercellular lipids between corneocytes [61] regulates the TEWL [62]. The decrease of skin hydration indicates the increase of skin TEWL and, at the same time, confirms the skin barrier dysfunction in AD patients [63,64].

During the four weeks of use, patients tolerated verum and placebo well, and no adverse events occurred, which can certify the safety of using extracts. However, the odor and fluidity of the cream preparation still needs to be improved. Since AD is a chronic disease requiring long-term management, the study period of four weeks was too short to determine the safety, furthermore, the small sample size limits the comparison of this extract with other topical drugs, such as topical corticosteroids and tacrolimus ointment.

The extracts used in this experiment are derived from apples, kale and green tea, and the edibility of the sources ensures that the extracts are natural, have very low risk of sensitization, and safety for long-term use because typical plant allergens such as sesquiterpene lactones, terpenes and polyacetylenes are not present in the extract [65]. It is frequently described that herbal, fruit or vegetable extracts are used in topical treatments [66–68]. Green tea extract contains high amounts of oligomeric proanthocyanidins such as epigallocatechin 3-gallate (EGCG)—a potent antioxidant with photo-protective properties. However, clinical studies are needed to determine if EGCG has a clinically relevant effect on AD lesions. About the topical application of curly kale and apple extract the reports are rare, but orally administered polyphenol-enriched apple extracts attenuated food allergy in mice [69]. Double blind clinical trials of Applephenon on pediatric patients with atopic dermatitis, and tests using type I allergic model mice suggested that Applephenon might regulate allergic reactions [70]. Wu et al., investigating the therapeutic role of phloretin in mouse model of allergic contact dermatitis found that AD-like symptoms were alleviated and immunopathological effects were reversed [71]. Curly kale extracts rich in carotenoids orally administered have shown to reduce radical formation in skin due to irradiation and protect the collagen in the dermis [72,73]. Furthermore, kaempferol reduces oxidative stress [74] and appears to be an effective topical wound healing agent alone or together with other flavonoids [75,76].To the best of our knowledge, the application of kaempferol extracted from curly kale has not been investigated on skin so far. In the extract used and in the verum formulation of the clinical trial L-arginine is also added. As a constituent of filaggrin, L-arginine can be processed into natural moisturizing factors in the skin [77]. L-arginine can repair the skin barrier from the following two aspects: L-arginine can be hydrolyzed by arginase I into ornithine and urea, which is well known, and can enhance the hydration of the stratum corneum and antimicrobial peptides [78]; L-arginine forms NO through NO synthase (NOS), NO can modulate inflammation, stimulate the proliferation of endothelial cells and re-epithelialization [79,80]. Thus, L-arginine could contribute to

improved skin hydration and the reduction of TEWL in the skin of the AD verum group in the clinical study.

The results shown in this paper agree with the findings mentioned in the literature but present for the first time the effect of the combined ingredients from different selected plants in vitro and in vivo after topical application on AD patients.

5. Conclusions

In conclusion, the extract applied has strong antioxidant capacity, anti-inflammatory effects, and at the same time increases the expression of skin barrier proteins. Components of the basic formula of the cream containing the extract or part of the extract can penetrate through the stratum corneum but the available experimental data are not sufficient to prove that they could reach other layers of the epidermis. The basic cream formulation still has room for improvement to achieve a higher transdermal penetration efficiency for the incorporated active ingredients. Nevertheless, the clinical study proved that the new extract significantly improves the clinical signs and symptoms in patients with mild to moderate AD. The treatment is well-tolerated, which may provide a new topical skin care product for AD patients.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox11061071/s1, Figure S1: Representative averaged Raman spectra of verum (a) and placebo (b) creams; Figure S2: Representative averaged Raman spectra of untreated skin (left column), placebo cream-treated skin (middle column) and verum cream-treated skin (right column) recorded at different exemplary skin depths (2, 8, 16 and 22 µm). Stratum corneum thickness is 18 µm after 2 hours penetration time.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All of the data is contained within the article and the supplementary materials.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Bylund, S.; Kobyletzki, L.B.; Svalstedt, M.; Svensson, Å. Prevalence and Incidence of Atopic Dermatitis: A Systematic Review. *Acta Derm.Venereol.* **2020**, *100*, adv00160. [CrossRef] [PubMed]
2. Munera-Campos, M.; Carrascosa, J.M. Innovation in Atopic Dermatitis: From Pathogenesis to Treatment. *Actas Derm. Sifiliogr.* **2020**, *111*, 205–221. [CrossRef]
3. Furue, M. Regulation of Filaggrin, Loricrin and Involucrin by IL-4, IL-13, IL-17A, IL-22, AHR, and NRF2: Pathogenic Implications in Atopic Dermatitis. *Int. J. Mol. Sci.* **2020**, *21*, 5382. [CrossRef] [PubMed]
4. Proksch, E.; Brandner, J.M.; Jensen, J.M. The skin: An indispensable barrier. *Exp. Dermatol.* **2008**, *17*, 1063–1072. [CrossRef] [PubMed]

5. Kim, B.E.; Leung, D.Y.M. Significance of Skin Barrier Dysfunction in Atopic Dermatitis. *Allergy Asthma Immunol. Res.* **2018**, *10*, 207–215. [CrossRef]
6. Martin, M.J.; Estravís, M.; García-Sánchez, A.; Dávila, I.; Isidoro-García, M.; Sanz, C. Genetics and Epigenetics of Atopic Dermatitis: An Updated Systematic Review. *Genes* **2020**, *11*, 442. [CrossRef]
7. Kezic, S.; Jakasa, I. Filaggrin and Skin Barrier Function. *Curr. Probl. Dermatol.* **2016**, *49*, 1–7. [CrossRef]
8. Furue, M.; Ulzii, D.; Vu, Y.H.; Tsuji, G.; Kido-Nakahara, M.; Nakahara, T. Pathogenesis of Atopic Dermatitis: Current Paradigm. *Iran. J. Immunol. IJI* **2019**, *16*, 97–107. [CrossRef]
9. Bertino, L.; Guarneri, F.; Cannavò, S.P.; Casciaro, M.; Pioggia, G.; Gangemi, S. Oxidative Stress and Atopic Dermatitis. *Antioxidants* **2020**, *9*, 196. [CrossRef] [PubMed]
10. van den Bogaard, E.H.; Bergboer, J.G.; Vonk-Bergers, M.; van Vlijmen-Willems, I.M.; Hato, S.V.; van der Valk, P.G.; Schröder, J.M.; Joosten, I.; Zeeuwen, P.L.; Schalkwijk, J. Coal tar induces AHR-dependent skin barrier repair in atopic dermatitis. *J. Clin. Investig.* **2013**, *123*, 917–927. [CrossRef]
11. Langan, S.M.; Irvine, A.D.; Weidinger, S. Atopic dermatitis. *Lancet* **2020**, *396*, 345–360. [CrossRef]
12. Wollenberg, A.; Barbarot, S.; Bieber, T.; Christen-Zaech, S.; Deleuran, M.; Fink-Wagner, A.; Gieler, U.; Girolomoni, G.; Lau, S.; Muraro, A.; et al. Consensus-based European guidelines for treatment of atopic eczema (atopic dermatitis) in adults and children: Par II. *J. Eur. Acad. Dermatol. Venereol. JEADV* **2018**, *32*, 850–878. [CrossRef] [PubMed]
13. Simpson, E.L.; Bruin-Weller, M.; Flohr, C.; Ardern-Jones, M.R.; Barbarot, S.; Deleuran, M.; Bieber, T.; Vestergaard, C.; Brown, S.J.; Cork, M.J.; et al. When does atopic dermatitis warrant systemic therapy? Recommendations from an expert panel of the International Eczema Council. *J. Am. Acad. Dermatol.* **2017**, *77*, 623–633. [CrossRef] [PubMed]
14. Barnes, L.; Kaya, G.; Rollason, V. Topical corticosteroid-induced skin atrophy: A comprehensive review. *Drug safety* **2015**, *38*, 493–509. [CrossRef]
15. Mandelin, J.; Remitz, A.; Reitamo, S. Effect of oral acetylsalicylic acid on burning caused by tacrolimus ointment in patients with atopic dermatitis. *Arch. Dermatol.* **2010**, *146*, 1178–1180. [CrossRef]
16. Eichenfield, L.F.; Tom, W.L.; Berger, T.G.; Krol, A.; Paller, A.S.; Schwarzenberger, K.; Bergman, J.N.; Chamlin, S.L.; Cohen, D.E.; Cooper, K.D.; et al. Guidelines of care for the management of atopic dermatitis: Section 2. Management and treatment of atopic dermatitis with topical therapies. *J. Am. Acad. Dermatol.* **2014**, *71*, 116–132. [CrossRef]
17. Wollenberg, A.; Barbarot, S.; Bieber, T.; Christen-Zaech, S.; Deleuran, M.; Fink-Wagner, A.; Gieler, U.; Girolomoni, G.; Lau, S.; Muraro, A.; et al. Consensus-based European guidelines for treatment of atopic eczema (atopic dermatitis) in adults and children: Part I. *J. Eur. Acad. Dermatol. Venereol. JEADV* **2018**, *32*, 657–682. [CrossRef]
18. Zessner, H.; Pan, L.; Will, F.; Klimo, K.; Knauft, J.; Niewöhner, R.; Hümmer, W.; Owen, R.; Richling, E.; Frank, N.; et al. Fractionation of polyphenol-enriched apple juice extracts to identify constituents with cancer chemopreventive potential. *Mol. Nutr. Food Res.* **2008**, *52*, S28–S44. [CrossRef]
19. Zhang, R.; Ai, X.; Duan, Y.; Xue, M.; He, W.; Wang, C.; Xu, T.; Xu, M.; Liu, B.; Li, C.; et al. Kaempferol ameliorates H9N2 swine influenza virus-induced acute lung injury by inactivation of TLR4/MyD88-mediated NF-κB and MAPK signaling pathways. *Biomed. Pharmacother. Biomed. Pharmacother.* **2017**, *89*, 660–672. [CrossRef]
20. Liu, C.; Liu, H.; Lu, C.; Deng, J.; Yan, Y.; Chen, H.; Wang, Y.; Liang, C.L.; Wei, J.; Han, L.; et al. Kaempferol attenuates imiquimod-induced psoriatic skin inflammation in a mouse model. *Clin. Exp. Immunol.* **2019**, *198*, 403–415. [CrossRef]
21. Matsuzaki, T.; Hara, Y. Antioxidative Activity of Tea Leaf Catechins. *Nippon Nōgeikagaku Kaishi* **1985**, *59*, 129–134. [CrossRef]
22. Nichols, J.A.; Katiyar, S.K. Skin photoprotection by natural polyphenols: Anti-inflammatory, antioxidant and DNA repair mechanisms. *Arch. Dermatol. Res.* **2010**, *302*, 71–83. [CrossRef]
23. Zhai, Y.; Dang, Y.; Gao, W.; Zhang, Y.; Xu, P.; Gu, J.; Ye, X. P38 and JNK signal pathways are involved in the regulation of phlorizin against UVB-induced skin damage. *Exp. Dermatol.* **2015**, *24*, 275–279. [CrossRef]
24. Bernard, F.X.; Morel, F.; Camus, M.; Pedretti, N.; Barrault, C.; Garnier, J.; Lecron, J.C. Keratinocytes under Fire of Proinflammatory Cytokines: Bona Fide Innate Immune Cells Involved in the Physiopathology of Chronic Atopic Dermatitis and Psoriasis. *J. Allergy* **2012**, *2012*, 718725. [CrossRef] [PubMed]
25. Roth, S.A.; Simanski, M.; Rademacher, F.; Schröder, L.; Harder, J. The pattern recognition receptor NOD2 mediates Staphylococcus aureus-induced IL-17C expression in keratinocytes. *J. Investig. Dermatol.* **2014**, *134*, 374–380. [CrossRef] [PubMed]
26. Meinke, M.C.; Haag, S.F.; Schanzer, S.; Groth, N.; Gersonde, I.; Lademann, J. Radical protection by sunscreens in the infrared spectral range. *Photochem. Photobiol.* **2011**, *87*, 452–456. [CrossRef] [PubMed]
27. Souza, C.; Maia Campos, P.; Schanzer, S.; Albrecht, S.; Lohan, S.B.; Lademann, J.; Darvin, M.E.; Meinke, M.C. Radical-Scavenging Activity of a Sunscreen Enriched by Antioxidants Providing Protection in the Whole Solar Spectral Range. *Skin Pharmacol. Physiol.* **2017**, *30*, 81–89. [CrossRef] [PubMed]
28. Caspers, P.J.; Lucassen, G.W.; Carter, E.A.; Bruining, H.A.; Puppels, G.J. In vivo confocal Raman microspectroscopy of the skin: Noninvasive determination of molecular concentration profiles. *J. Investig. Dermatol.* **2001**, *116*, 434–442. [CrossRef] [PubMed]
29. Choe, C.; Lademann, J.; Darvin, M.E. Analysis of Human and Porcine Skin in vivo/ex vivo for Penetration of Selected Oils by Confocal Raman Microscopy. *Skin Pharmacol. Physiol.* **2015**, *28*, 318–330. [CrossRef]
30. Lohan, S.B.; Saeidpour, S.; Solik, A.; Schanzer, S.; Richter, H.; Dong, P.; Darvin, M.E.; Bodmeier, R.; Patzelt, A.; Zoubari, G.; et al. Investigation of the cutaneous penetration behavior of dexamethasone loaded to nano-sized lipid particles by EPR spectroscopy, and confocal Raman and laser scanning microscopy. *Eur. J. Pharm. Biopharm.* **2017**, *116*, 102–110. [CrossRef]

31. Darvin, M.E.; Meinke, M.C.; Sterry, W.; Lademann, J. Optical methods for noninvasive determination of carotenoids in human and animal skin. *J. Biomed. Opt.* **2013**, *18*, 61230. [CrossRef]
32. Chopra, R.; Vakharia, P.P.; Sacotte, R.; Patel, N.; Immaneni, S.; White, T.; Kantor, R.; Hsu, D.Y.; Silverberg, J.I. Severity strata for Eczema Area and Severity Index (EASI), modified EASI, Scoring Atopic Dermatitis (SCORAD), objective SCORAD, Atopic Dermatitis Severity Index and body surface area in adolescents and adults with atopic dermatitis. *Br. J. Dermatol.* **2017**, *177*, 1316–1321. [CrossRef] [PubMed]
33. Severity scoring of atopic dermatitis: The SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* **1993**, *186*, 23–31. [CrossRef]
34. du Plessis, J.; Stefaniak, A.; Eloff, F.; John, S.; Agner, T.; Chou, T.C.; Nixon, R.; Steiner, M.; Franken, A.; Kudla, I.; et al. International guidelines for the in vivo assessment of skin properties in non-clinical settings: Part 2. transepidermal water loss and skin hydration. *Skin Res. Technol.* **2013**, *19*, 265–278. [CrossRef] [PubMed]
35. Piérard, G.E. EEMCO guidance for the assessment of skin colour. *J. Eur. Acad. Dermatol. Venereol. JEADV* **1998**, *10*, 1–11. [CrossRef]
36. Eichenfield, L.F.; Ahluwalia, J.; Waldman, A.; Borok, J.; Udkoff, J.; Boguniewicz, M. Current guidelines for the evaluation and management of atopic dermatitis: A comparison of the Joint Task Force Practice Parameter and American Academy of Dermatology guidelines. *J. Allergy Clin. Immunol.* **2017**, *139*, S49–S57. [CrossRef]
37. Mohan, G.C.; Lio, P.A. Comparison of Dermatology and Allergy Guidelines for Atopic Dermatitis Management. *JAMA Dermatol.* **2015**, *151*, 1009–1013. [CrossRef]
38. Weidinger, S.; Baurecht, H.; Schmitt, J. A 5-year randomized trial on the safety and efficacy of pimecrolimus in atopic dermatitis: A critical appraisal. *Br. J. Dermatol.* **2017**, *177*, 999–1003. [CrossRef]
39. Ji, H.; Li, X.K. Oxidative Stress in Atopic Dermatitis. *Oxidative Med. Cell. Longev.* **2016**, *2016*, 2721469. [CrossRef]
40. Chung, J.; Oh, S.Y.; Shin, Y.K. Association of glutathione-S-transferase polymorphisms with atopic dermatitis risk in preschool age children. *Clin. Chem. Lab. Med.* **2009**, *47*, 1475–1481. [CrossRef]
41. Ahn, K. The role of air pollutants in atopic dermatitis. *J. Allergy Clin. Immunol.* **2014**, *134*, 993–999. [CrossRef] [PubMed]
42. Niwa, Y.; Sumi, H.; Kawahira, K.; Terashima, T.; Nakamura, T.; Akamatsu, H. Protein oxidative damage in the stratum corneum: Evidence for a link between environmental oxidants and the changing prevalence and nature of atopic dermatitis in Japan. *Br. J. Dermatol.* **2003**, *149*, 248–254. [CrossRef] [PubMed]
43. Haag, S.F.; Tscherch, K.; Arndt, S.; Kleemann, A.; Gersonde, I.; Lademann, J.; Rohn, S.; Meinke, M.C. Enhancement of skin radical scavenging activity and stratum corneum lipids after the application of a hyperforin-rich cream. *Eur. J. Pharm. Biopharm.* **2014**, *86*, 227–233. [CrossRef] [PubMed]
44. Darvin, M.E.; Fluhr, J.W.; Schanzer, S.; Richter, H.; Patzelt, A.; Meinke, M.C.; Zastrow, L.; Golz, K.; Doucet, O.; Sterry, W.; et al. Dermal carotenoid level and kinetics after topical and systemic administration of antioxidants: Enrichment strategies in a controlled in vivo study. *J. Dermatol. Sci.* **2011**, *64*, 53–58. [CrossRef] [PubMed]
45. Lademann, J.; Vergou, T.; Darvin, M.E.; Patzelt, A.; Meinke, M.C.; Voit, C.; Papakostas, D.; Zastrow, L.; Sterry, W.; Doucet, O. Influence of Topical, Systemic and Combined Application of Antioxidants on the Barrier Properties of the Human Skin. *Skin Pharmacol. Physiol.* **2016**, *29*, 41–46. [CrossRef] [PubMed]
46. Lademann, J.; Martschick, A.; Kluschke, F.; Richter, H.; Fluhr, J.W.; Patzelt, A.; Jung, S.; Chekerov, R.; Darvin, M.E.; Haas, N.; et al. Efficient prevention strategy against the development of a palmar-plantar erythrodysesthesia during chemotherapy. *Skin Pharmacol. Physiol.* **2014**, *27*, 66–70. [CrossRef]
47. Choe, C.; Lademann, J.; Darvin, M.E. A depth-dependent profile of the lipid conformation and lateral packing order of the stratum corneum in vivo measured using Raman microscopy. *Analyst* **2016**, *141*, 1981–1987. [CrossRef]
48. Mujica Ascencio, S.; Choe, C.; Meinke, M.C.; Müller, R.H.; Maksimov, G.V.; Wigger-Alberti, W.; Lademann, J.; Darvin, M.E. Confocal Raman microscopy and multivariate statistical analysis for determination of different penetration abilities of caffeine and propylene glycol applied simultaneously in a mixture on porcine skin ex vivo. *Eur. J. Pharm. Biopharm.* **2016**, *104*, 51–58. [CrossRef]
49. Jung, N.; Namjoshi, S.; Mohammed, Y.; Grice, J.E.; Benson, H.A.E.; Raney, S.G.; Roberts, M.S.; Windbergs, M. Application of Confocal Raman Microscopy for the Characterization of Topical Semisolid Formulations and their Penetration into Human Skin Ex Vivo. *Pharm. Res.* **2022**. [CrossRef]
50. Franzen, L.; Selzer, D.; Fluhr, J.W.; Schaefer, U.F.; Windbergs, M. Towards drug quantification in human skin with confocal Raman microscopy. *Eur. J. Pharm. Biopharm.* **2013**, *84*, 437–444. [CrossRef]
51. Mitamura, Y.; Nunomura, S.; Furue, M.; Izuhara, K. IL-24: A new player in the pathogenesis of pro-inflammatory and allergic skin diseases. *Allergol. Int.* **2020**, *69*, 405–411. [CrossRef] [PubMed]
52. Kamsteeg, M.; Zeeuwen, P.L.; de Jongh, G.J.; Rodijk-Olthuis, D.; Zeeuwen-Franssen, M.E.; van Erp, P.E.; Schalkwijk, J. Increased expression of carbonic anhydrase II (CA II) in lesional skin of atopic dermatitis: Regulation by Th2 cytokines. *J. Investig. Dermatol.* **2007**, *127*, 1786–1789. [CrossRef] [PubMed]
53. Suri, B.K.; Verma, N.K.; Schmidtchen, A. Toll-like Receptor 3 Agonist, Polyinosinic-polycytidylic Acid, Upregulates Carbonic Anhydrase II in Human Keratinocytes. *Acta Derm. Venereol.* **2018**, *98*, 762–765. [CrossRef] [PubMed]
54. Blome, C.; Radtke, M.A.; Eissing, L.; Augustin, M. Quality of Life in Patients with Atopic Dermatitis: Disease Burden, Measurement, and Treatment Benefit. *Am. J. Clin. Dermatol.* **2016**, *17*, 163–169. [CrossRef]

55. Yosipovitch, G.; Fleischer, A. Itch associated with skin disease: Advances in pathophysiology and emerging therapies. *Am. J. Clin. Dermatol.* **2003**, *4*, 617–622. [CrossRef]
56. Damien, F.; Boncheva, M. The extent of orthorhombic lipid phases in the stratum corneum determines the barrier efficiency of human skin in vivo. *J. Investig. Dermatol.* **2010**, *130*, 611–614. [CrossRef]
57. Eberlein-König, B.; Schäfer, T.; Huss-Marp, J.; Darsow, U.; Möhrenschlager, M.; Herbert, O.; Abeck, D.; Krämer, U.; Behrendt, H.; Ring, J. Skin surface pH, stratum corneum hydration, trans-epidermal water loss and skin roughness related to atopic eczema and skin dryness in a population of primary school children. *Acta Derm. Venereol.* **2000**, *80*, 188–191. [CrossRef]
58. Blank, I.H.; Shappirio, E.B. The water content of the stratum corneum. III. Effect of previous contact with aqueous solutions of soaps and detergents. *J. Investig. Dermatol.* **1955**, *25*, 391–401. [CrossRef]
59. Choe, C.; Schleusener, J.; Lademann, J.; Darvin, M.E. Keratin-water-NMF interaction as a three layer model in the human stratum corneum using in vivo confocal Raman microscopy. *Sci. Rep.* **2017**, *7*, 15900. [CrossRef]
60. Nakazawa, H.; Ohta, N.; Hatta, I. A possible regulation mechanism of water content in human stratum corneum via intercellular lipid matrix. *Chem. Phys. Lipids* **2012**, *165*, 238–243. [CrossRef]
61. van Smeden, J.; Janssens, M.; Gooris, G.S.; Bouwstra, J.A. The important role of stratum corneum lipids for the cutaneous barrier function. *Biochim. Biophys. Acta* **2014**, *1841*, 295–313. [CrossRef] [PubMed]
62. Long, S.A.; Wertz, P.W.; Strauss, J.S.; Downing, D.T. Human stratum corneum polar lipids and desquamation. *Arch. Dermatol. Res.* **1985**, *277*, 284–287. [CrossRef] [PubMed]
63. Pavlis, J.; Yosipovitch, G. Management of Itch in Atopic Dermatitis. *Am. J. Clin. Dermatol.* **2018**, *19*, 319–332. [CrossRef] [PubMed]
64. Janssens, M.; van Smeden, J.; Gooris, G.S.; Bras, W.; Portale, G.; Caspers, P.J.; Vreeken, R.J.; Hankemeier, T.; Kezic, S.; Wolterbeek, R.; et al. Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. *J. Lipid Res.* **2012**, *53*, 2755–2766. [CrossRef] [PubMed]
65. Schempp, C.M.; Schöpf, E.; Simon, J.C. Plant-induced toxic and allergic dermatitis (phytodermatitis). *Der Hautarzt; Zeitschrift fur Dermatologie, Venerologie, und verwandte Gebiete* **2002**, *53*, 93–97. [CrossRef] [PubMed]
66. Hoffmann, J.; Gendrisch, F.; Schempp, C.M.; Wölfle, U. New Herbal Biomedicines for the Topical Treatment of Dermatological Disorders. *Biomedicines* **2020**, *8*, 27. [CrossRef]
67. Zink, A.; Traidl-Hoffmann, C. Green tea in dermatology–myths and facts. *J. Ger. Soc. Dermatol. JDDG* **2015**, *13*, 768–775. [CrossRef]
68. Hwang, Y.; Chang, B.; Kim, T.; Kim, S. Ameliorative effects of green tea extract from tannase digests on house dust mite antigen-induced atopic dermatitis-like lesions in NC/Nga mice. *Arch. Dermatol. Res.* **2019**, *311*, 109–120. [CrossRef]
69. Zuercher, A.W.; Holvoet, S.; Weiss, M.; Mercenier, A. Polyphenol-enriched apple extract attenuates food allergy in mice. *Clin. Exp. Allergy* **2010**, *40*, 942–950. [CrossRef]
70. Akazome, Y. Characteristics and physiological functions of polyphenols from apples. *BioFactors* **2004**, *22*, 311–314. [CrossRef]
71. Wu, C.S.; Lin, S.C.; Li, S.; Chiang, Y.C.; Bracci, N.; Lehman, C.W.; Tang, K.T.; Lin, C.C. Phloretin alleviates dinitrochlorobenzene-induced dermatitis in BALB/c mice. *Int. J. Immunopathol. Pharmacol.* **2020**, *34*, 2058738420929442. [CrossRef]
72. Meinke, M.C.; Nowbary, C.K.; Schanzer, S.; Vollert, H.; Lademann, J.; Darvin, M.E. Influences of Orally Taken Carotenoid-Rich Curly Kale Extract on Collagen I/Elastin Index of the Skin. *Nutrients* **2017**, *9*, 775. [CrossRef] [PubMed]
73. Meinke, M.C.; Friedrich, A.; Tscherch, K.; Haag, S.F.; Darvin, M.E.; Vollert, H.; Groth, N.; Lademann, J.; Rohn, S. Influence of dietary carotenoids on radical scavenging capacity of the skin and skin lipids. *Eur. J. Pharm. Biopharm.* **2013**, *84*, 365–373. [CrossRef] [PubMed]
74. Sekiguchi, A.; Motegi, S.I.; Fujiwara, C.; Yamazaki, S.; Inoue, Y.; Uchiyama, A.; Akai, R.; Iwawaki, T.; Ishikawa, O. Inhibitory effect of kaempferol on skin fibrosis in systemic sclerosis by the suppression of oxidative stress. *J. Dermatol. Sci.* **2019**, *96*, 8–17. [CrossRef] [PubMed]
75. Özay, Y.; Güzel, S.; Yumrutaş, Ö.; Pehlivanoğlu, B.; Erdoğdu, İ.H.; Yildirim, Z.; Türk, B.A.; Darcan, S. Wound Healing Effect of Kaempferol in Diabetic and Nondiabetic Rats. *J. Surg. Res.* **2019**, *233*, 284–296. [CrossRef]
76. Al-Madhagy, S.A.; Mostafa, N.M.; Youssef, F.S.; Awad, G.E.A.; Eldahshan, O.A.; Singab, A.N.B. Metabolic profiling of a polyphenolic-rich fraction of Coccinia grandis leaves using LC-ESI-MS/MS and in vivo validation of its antimicrobial and wound healing activities. *Food Funct.* **2019**, *10*, 6267–6275. [CrossRef]
77. Candi, E.; Schmidt, R.; Melino, G. The cornified envelope: A model of cell death in the skin. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 328–340. [CrossRef]
78. Grether-Beck, S.; Felsner, I.; Brenden, H.; Kohne, Z.; Majora, M.; Marini, A.; Jaenicke, T.; Rodriguez-Martin, M.; Trullas, C.; Hupe, M.; et al. Urea uptake enhances barrier function and antimicrobial defense in humans by regulating epidermal gene expression. *J. Investig. Dermatol.* **2012**, *132*, 1561–1572. [CrossRef]
79. Seifter, E.; Rettura, G.; Barbul, A.; Levenson, S.M. Arginine: An essential amino acid for injured rats. *Surgery* **1978**, *84*, 224–230.
80. Blecher, K.; Martinez, L.R.; Tuckman-Vernon, C.; Nacharaju, P.; Schairer, D.; Chouake, J.; Friedman, J.M.; Alfieri, A.; Guha, C.; Nosanchuk, J.D.; et al. Nitric oxide-releasing nanoparticles accelerate wound healing in NOD-SCID mice. *Nanomed. Nanotechnol. Biol. Med.* **2012**, *8*, 1364–1371. [CrossRef]

Curriculum Vitae

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Publication list

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